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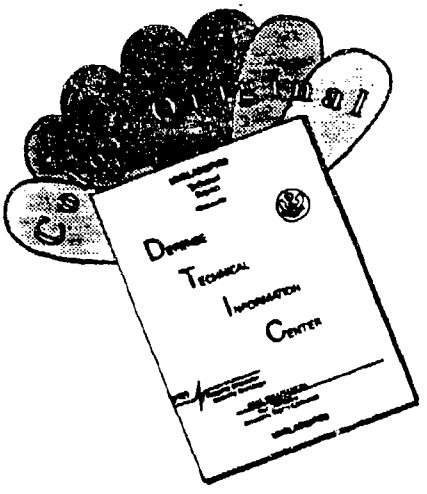

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FOREWORD

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Walter H. Adey, 18 Jun 96
Principal Investigator

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Executive Summary

This Rapid Bioassay of Surface Waters based on macrophytic aquatic plants was developed by the Smithsonian Institution with funding from the Department of Defense Legacy Program. It provides a practical and low cost system for identifying the degradation of streams on DOD bases within the Chesapeake Bay Watershed and is applicable to determining diffuse source pollution throughout the entire watershed.

The Chesapeake Bay Watershed encompasses 64,000 square miles of land across six states with more than 100,000 miles of streams and rivers. Many of the streams in this area are not in good health, and have lost or are losing their self-purifying characteristics. Unfortunately, due to the combined lower quality flow from thousands of streams, diffuse source pollution is continuing to degrade the major waterways and the Bay itself. This degradation continues in spite of the intensive Federal, state and local efforts to remove point sources of pollution and to improve these larger bodies of water. The project described herein is developing the techniques that would identify the weakening of the "first line of defense," the aquatic plants, in a stream's natural ability to handle the increasing introduction of contaminants.

Bioindicators such as fish abundance, benthic (bottom dwelling) macroinvertebrate populations and diatoms have long been used by scientists as a means of assessing the quality of aquatic ecosystems. Stream surveys utilizing these methods have been limited primarily to

scientists who are experts in these fields. Costs tend to be high and they limit our ability to quickly survey thousands of miles of streams.

The rapid bioassay described in this report utilizes aquatic higher plants that are easily recognized and counted in the field. Sophisticated, analytical techniques, adaptable for software use by volunteers or workers with minimum training, have been developed for converting this simplified data base to stream quality indices.

In this project: 1) a working geomorphological framework for the Chesapeake Bay Watershed, providing a series of provinces within which the bioassays can be performed with maximum precision, has been developed; 2) the criteria for selecting reference or baseline streams of high and low quality and back-checking their veracity has been established; 3) field techniques and a working field manual have been developed; 4) mathematical techniques for assessing macrophyte density and diversity and for determining stream water quality have been developed at two levels: a.) a very rapid bioassay based on eleven indicator species, and b.) a rapid bioassay based on a combination of diversity measures; 5) the reference stream field work and data set have been completed for three of the five geomorphological provinces. In this report, we describe the results and analytical procedure as specifically applied to the Coastal Plain and Piedmont Provinces within which lie virtually all of the DOD bases of the region. During the coming warm season all accessible streams on region DOD bases will be rated utilizing the methodology described in this report.

The leadership of the DOD through this program is enabling us to produce a rapid bioassay technique that can be used to identify the water quality of the many streams on DOD bases. In addition, this research provides a realistic and high quality methodology that can be performed by state surveys, or an interested public, on the thousands of streams that make up the Chesapeake Bay Watershed. It is partly by this means that the very serious problem of diffuse source pollution can be recognized and solved on a local basis.

INTRODUCTION

Background

For millennia, fresh water streams have been used by human communities as a convenient device for flushing away wastes, thereby increasing the carrying capacity of the local terrain. This worked well until large scale commercial farming and the industrial revolution allowed unprecedented population densities.

The intense concentration of people in the 19th and 20th centuries, and the ability of modern populations to collect and concentrate chemical elements and compounds at both the landscape and the biosphere scale, as well as to physically modify entire watersheds, has greatly altered the status of a very high percentage of the earth's streams. The industrial development of entirely new chemical compounds, many of which are toxic or carcinogenic, and are routinely available on farms and in homes, has considerably aggravated control of water pollution.

The larger waterways of the United States reached a critical point forty years ago, particularly in heavily populated areas, when they had become polluted to the point of being aesthetically obnoxious and dangerous to public health. Some were effectively dead. Since that

time, a great deal of effort has been placed on identification and removal of the major point sources of pollution on most of our national waters. This process involves primarily the building of more effective sewage treatment plants and the prevention of the use of streams for the disposal of large scale industrial wastes. The practical elimination of many large point-sources of pollution led to a remarkable rejuvenation in the 1960's, 70's and 80's, bringing life back to biologically dead streams, lakes and rivers.

During this period of general improvement, however, the population continued to grow, as did its standard of living, at least as measured by the processing of materials. Unfortunately, the secondary level of treatment offered by most sewage treatment plants is now becoming inadequate to protect the estuaries, bays and lakes into which rivers empty. Even more critical at the present time, the cumulative effect of numerous non-point sources of pollution, including those from farming and urban/suburban runoff, has caused our progress to plateau and in many cases reverse. A more diffuse, but still massive level of pollution is now responsible for the continued degradation of all our waterways.

A pollutant addition to a stream has the ability to travel hundreds to thousands of miles, combining with pollutants from countless other streams, into large and economically important bodies of water. In the past decade, we have begun to see significant pollution of groundwater and the ultimate aqueous sink on our planet, the oceans (see eg. Lange and Lambert, 1994, re: elevated chlorinated pesticides in whales, dolphins and grey seals). It is clear that a major

national priority must be the identification and removal of diffuse pollution at the level of our small streams and wetlands.

It has been recognized that the nation's largest estuary, the Chesapeake Bay, is endangered. A Federal mandate has been issued to reverse the trend of degradation. The need for identification and reduction of non-point source or minor point source pollution loads from the Chesapeake Bay's 64,000 square mile watershed has been accepted. The task of identifying pollution on thousands of miles of small streams must be a major element of planning for future clean-up. This task is underway, but it has proven quite expensive and time consuming using available standard methods. This study is laying the framework for a faster, more cost-effective program for the analysis of stream water quality. The military bases of the Chesapeake Bay Watershed provide ideal test and development systems because they are numerous and diverse and yet are far more controllable than public areas.

A flowing stream or river, in good health, is self purifying. The natural bacteria, protozoa and fungi, particularly when abundant submergent and emergent plant (macrophyte) surface is present for their residence, breakdown many organic compounds, releasing carbon dioxide and nutrients. The higher levels of oxygen produced by a healthy photosynthesizing stream are conducive to the breakdown of many of the most difficult of synthetic chemicals (Adey et al, 1995). Algae, mosses and higher plants remove the nutrients that would then unbalance or eutrophicate the ecosystem, locking them up in their tissues (Carter et al, 1988; Seidel, 1976; McNabb et al, 1970) and eventually delivering them, as components of particulates, to sediment

burial sites. These plants also uptake a wide variety of potentially poisonous compounds and elements, including heavy metals, and either detoxify them or lock them up for eventual delivery to the sediments of a deep water sink. In some cases, at low levels, many potentially polluting elements, ions and compounds actually supplement limiting growth resources for macrophytes and allow an increase in biodiversity and biomass.

As the quantity, consistency and combination of pollution levels begin to increase in streams, sensitive species of plants and animals are lost. Eventually, with further pollution, community structure changes and diversity falls across a broad spectrum. Some tolerant species may actually increase in abundance at moderate pollution levels, and biomass and productivity may increase as well. However, as loads increase further, more tolerant species are lost, and the biomass of higher plants and animals begins to fall. Finally, depending upon the nature of the pollution problem, oxygen levels fall and eventually only specialized bacteria remain in an environment that cannot support higher life.

While technically, analytical chemistry is the primary tool for identification and quantification of pollution problems, in practice it is simply too expensive, time consuming and time limited for many search and mapping operations. Also there are more than 1,500 pollutants released into the aquatic environment, while routine water testing applies only to 30-40 parameters (Mason, 1990). Furthermore, as has been repeatedly demonstrated, levels of a chemical pollutant that are so low that they are difficult and expensive to routinely detect, can be concentrated in food webs to the level of driving species to extinction and poisoning human

populations. Even the routine and le lly accepted release of macronutrients, such as phosphorous, which are easily and cheaply detected, can lead to massive unbalancing of enormous areas of waterways.

A standard of detection utilizing biological effects, a "bioassay," rather than chemical concentrations, the "safe level" of which is often either unknown or set unrealistically too high, is much more appropriate. Once a body of water has been identified as degraded by biological or ecological methods, the techniques of analytical chemistry can help determine the nature, severity and time spectrum of the polluting vectors. The sources of these problem pollutants can then be located and hopefully ameliorated.

Living organisms respond to pollution in complex ways. Some are highly sensitive. Others may even benefit due to the removal of competition, or due to the presence of a compound or element which is useable at low concentrations, but to ic at high levels. Nevertheless, theoretically the response of organisms can be used in a bioassay of the health of a body of water. A tremendous scientific effort has been directed to developing an understanding of how some small invertebrates, especially insects, some algae (diatoms), and fish, respond to pollution. It would be extremely valuable to have enough understanding of these "indicator species" that their presence, and/or their abundance, could demonstrate the presence of a particular pollutant. Numerous studies have been carried out in recent years to develop indicator species for specific pollutants (reviewed by Lange and Lambert, 1994).

While many of these techniques are valuable once the potential for a specific pollutant to occur in a specific locality is known, most are not amenable to searches for unknown diffuse pollution (Patrick and Palavage, 1994). Wild ecosystems are not like a controlled laboratory environment in which a rat or guinea pig can be used as a bioassay. In a specific aquatic ecosystem constant variation is a fact of life. Even without the effects of human activity, changes occur due to weather, seasons and internal species interactions. To develop accurate bioassays of living ecosystems, the researcher must deal with extreme complexity and ultimately the loss of that complexity due to perturbating and toxic factors.

In the report that follows, we first review the stream bioassay methods that have been heavily researched in the past several decades. We will also discuss their advantages and disadvantages. Finally, we introduce aquatic macrophytes, a major group of stream-based higher plants that have been largely ignored as a potential bioassay, at least in the fresh water bodies of North America. The body of this report presents an investigation of the bioassay potential of aquatic macrophytes as applied to the Chesapeake Bay Watershed.

Status of Existing Aquatic Bioassays

Many organism bioassay techniques have been developed that potentially allow identification and integration of sometimes complex pollution loadings (Plafkin et al, 1989; Hawkes, 1978; Pinder and Far, 1987). The organisms most frequently used for water quality assessment have been macroinvertebrates (particularly insects and their larvae), fish, and algae

(mostly diatoms). The methods that have been devised to use these organisms have many advantages and disadvantages, which are briefly discuss below. Unfortunately, a drawback of all of the older methods is that little effort has been made to standardize sampling methods within each type of bioassay technique (Patrick and Palavage, 1994).

The most extensively used method of determining water quality includes the quantitative and qualitative bioassay of benthic macroinvertebrates. Although some efforts have been made to include annelids, copepods and bryozoans, these bioassays are based primarily on insects and their larvae. Early approaches, in the 1960s, concentrated on qualitative analysis of the macroinvertebrate community, using indicator species to identify environmental stresses. In the 1970s, when it became generally recognized that this approach was very imprecise, the switch to quantitative methods began. In more recent approaches, taxonomic richness and diversity indices have been widely employed Now, with a large bank of data available in the literature, the emphasis is on an abbreviated method that has the reliability of a quantitative approach, but the simplicity and low cost of a qualitative approach. For example, Patrick and Palavage, 1994, published an extensive list of the tolerant/intolerant species that included invertebrates (macro and micro), algae and fish. It was hoped that this information would help return biomonitoring to a mixed quantitative/qualitative approach with "rapid assessment" methods.

Some would argue that overall, macroinvertebrate bioassays are simple, reliable and cost effective. The fauna is diverse, non-mobile, easily collected and most species have life cycles long enough that they can be reliably collected over a long warm season (Hilsenhoff,

1977, 1982). Extensive literature is available with large numbers of examples of specific tolerances of certain species to different environmental stresses, such as toxicity, oxygen depletion, or organic enrichment.

However, the arguments against macroinvertebrate bioassays are also very strong. For example, it is generally thought that qualitative methods (indicator species) are considerably less reliable than taxonomic richness or diversity indices (Lenat, 1988; Hilsenhoff, 1982). Yet the reliability of the quantitative methods often depends on a large sample size which is identified to the taxonomic level of species (Resh, 1975). Identification of large samples to the species level requires experienced entomologists and is very time-consuming. This has been the reason for the high costs associated with water quality analysis by macroinvertebrate bioassay (Lenat, 1988; Resh, 1984).

A simplified method, based on macroinvertebrates, has been developed for use by volunteer groups. This method is based on identification to the level of order. Unfortunately, this does not take into account a tremendous variation that occurs with regard to water quality and tolerance at the genus and species level (Lamp, 1994). Identification of many macroinvertebrates even to the generic level is time consuming and extremely difficult even for experts (Lamp, 1994). An additional drawback to this method is the extensive time required to survey just one site within a stream.

Many decisions regarding sampling technique and location must be made and can bias the results of any macroinvertebrate bioassay. Since macroinvertebrates are dependent on substrate and current, there are only certain points in the stream course which will yield adequate samples for analysis (Hilsenhoff, 1982). Some streams do not even have enough flow to support a substantial community. Most methods require that the sampler choose an appropriate site for sampling. In such cases, the experience and knowledge of the sampler will have an effect on the sample collected (Hilsenhoff, 1982).

New "rapid assessment" methods depend on multiple measures of a sample. Most include some measure based on environmental tolerances of organisms, such as a biotic index or numbers of "tolerant" species (Rosenberg and Resh, 1993). These methods do not employ diversity indices which are calculated below the level of genus. Therefore, they sacrifice a great deal of accuracy. Furthermore, they are still dependent on the skills and knowledge of experienced entomologists and require at least three to four days to produce results (Eaton and Lenat, 1991).

Fish have been less extensively used, although some researchers have maintained that a fish's "position at the top of the food chain helps provide an integrative view of the watershed environment" (Karr, 1981). However, in a 1994 study, it was determined that although fish do provide some information about the suitability of their overall environment, they do not clearly differentiate the differences in specific areas (Patrick and Palavage, 1994). Furthermore, the results indicated that because fish indicators tended not to be as sensitive to degraded conditions

as other groups, using the occurrence of any one species or group of species was not usually a good measure of water quality.

A further disadvantage noted by Patrick and Palavage in using fish fauna as indicators of water quality was the lack of literature on the subject. They found that rating fish as to their pollution tolerance was difficult without sufficient backup data. Just as with macroinvertebrates, problems occur with the sampling of fish in the field. Particularly because of the great mobility of fish, their sampling is more costly and less time efficient than other species sampling methods. (Karr, 1981).

Algae have been used as biomonitoring tools for quite some time (Kolkwitz and Marsson, 1908; Hentschel, 1925; Nauman, 1925; Butcher, 1947 and Liebmann, 1942). About ninety percent of the algae now utilized in this method are diatoms. Also considered are a few additional groups of equivalently microscopic algae such as blue greens and scenedesmids (Patrick and Palavage, 1994). The collection and identification of all of these groups require a considerable infrastructure of field and laboratory equipment, preparation and culturing systems and an adequate herbarium. Kolkwitz and Marsson first developed a system of zones based on the extent of degradation in the water quality. The pollution tolerance of certain species of algae were defined by their presence in a specific zone (Patrick, 1973). There were some flaws in this system in that some species classified as characteristic of a particular zone or condition may or may not occur there and may actually appear elsewhere (Hentschel, 1925; Nauman, 1932; Butcher, 1947 and Liebman, 1951). Additionally, as Butcher realized, certain species of algae

are tolerant of pollutants other than elevated organic levels and can actually flourish in these conditions.

Much information on the tolerance of pollution by algae is available, but considerable time and expertise are required for enumeration and identification of the algae. Although they enable monitoring of water quality from field samples, algae are also subject to a host of physical environmental factors (turbidity, light, temperature, etc.) that affects any given species' ability to compete with another species (Patrick, 1973). These additional parameters make it difficult to determine, within the functions of a natural ecosystem, the effect of a particular contaminant on an individual species of alga (Patrick, 1973; Boyle, 1984). Virtually all algal species used require a compound microscope for identification.

The effects of toxic chemicals on many algae have been measured by using field and laboratory evaluations. The field methods, particularly for benthic diatoms, generally involve collection on a previously established and standard artificial substrate. Results are analyzed based on indicator species or the dominance of a particular species (Butcher, 1947). The diatom research group at the Academy of Natural Sciences in Philadelphia mounted similar slides in an instrument called a diatometer and found that the communities that developed on the slide were similar to the benthic community found in traditional substrate sampling. Communities found on the slides also included those species common in the planktonic diatom community. Further studies showed that the structure of the diatom community, as well as the kinds of species and

total biomass, need to be examined in order to have a balanced analysis of pollution effects on diatom communities (Patrick, Roberts, and Davis, 1968).

Several physiological approaches to water quality have been developed using microscopic algae. The bio-stimulation approach is primarily used for evaluating the nutrient status of a particular waterway. Species most often used are Selenastrum capricornutum, Asterionella formosa, and Microcystis aeruginosa. The Standard Bottle Test (EPA, 1971) is a laboratory procedure that measures the specific growth rate or the maximum standing crop. Results are obtained by determining dry weight, by counting cells, by chlorophyll measurement or by total cell carbon based on C^{14} uptake (Mason, 1990). A continuous flow technique for bioassaying diatoms is also used (Watts & Harvey, 1963). These treatments are time consuming and require specialized equipment and expertise.

The diversity of algal species in clean water can be quite variable (Archibald 1972). Heavily polluted environments tend to have communities that are low in species diversity because sensitive species begin to die out as pollution levels increase. On the other hand, tolerant species will actually flourish under low levels of organic pollution, in part due to reduced competition, and if conditions are not severe, many sensitive species will remain. As a result, even mildly polluted rivers or streams can still have a high diversity (Mason, 1991). Therefore, the community structure of species must typically be examined as well as their individual tolerances.

The use of selected species of microalgae as indicators requires careful attention to the sampling process, became a single stream or river bed is composed of a wide variety of benthic and planktonic algal communities. Each of these communities shows their own array of species that can relate to microenvironmental factors based on the microscopic scale of the organisms being used. Each species in turn has its own tolerance or sensitivity to certain pollutants and a separate interaction with other species in its community. Patrick (1973) has stated after conducting numerous studies of diatoms, that the kinds of species found in the diatom communities vary greatly over time with no known change in the water quality and that these changes are the result of some environmental factors other than pollution. This questions the reliability of bioassays based on diatoms.

Aquatic Macrophytes

Most shallow, fresh water habitats have a complex array of flowering plants that have adapted to the aquatic environment. Both monocots and dicots are included, and some plant families are rich in or dominated by aquatic plant species (see eg. Godfrey and Wooten, 1979, 1981; Sculthorpe, 1967). In eastern North America, approximately 6,100 species of flowering plants from more than 70 families can be classed as aquatic and likely to occur in streams and their adjacent wetlands. The term macrophyte is used in aquatic environments to distinguish vascular plants, which are mostly flowering species, from the algae which are also ubiquitous but can generally be identified only with the use of microscope equipment.

In slow-moving to moderate-flow high quality streams of coastal and piedmont environments, flowering aquatic plants are usually important structuring elements. Simply removing these species from a stream is likely to radically alter the stream environment and its animal components. Thus, particularly in smaller streams, the recipients of most non-point source pollution, aquatic flowering plants are extremely important physically structuring elements of the community. Macrophytes are also primary producers and provide food or detritus for the base of the food web. Many produce oxygen, lock up nutrients and provide substrate and surface for microbes. All of these processes are key elements in the amelioration of the effects of pollution.

Studies have shown that changes in the composition of invertebrate communities have been profoundly affected following changes in the abundance and morphological type of higher plants, particularly submerged aquatic plants (Swartz et al, 1984). Although both invertebrates and plants are sensitive to pollutants and environmental changes, plants affect, and in some instances, create the quality of environment in which the invertebrates dwell.

It is therefore difficult to understand why so little emphasis has been placed on aquatic plant bioassays, particularly since the species involved are mostly large, easily counted and in a majority of the cases, relatively easy to identify. It is true that in much of North America, plant bioassay surveys can be carried out only during the 6 - 9 months of the warm season, but as Lenat and Barbour (1993) point out: "Seasonal changes of macroinvertebrate fauna are a major headache for routine water quality monitoring."

Biological monitoring using aquatic macrophytes, while almost unknown in North America, has been developing in the British Isles and on the European Continent over the past two decades (Caffrey, 1985; Haslam, 1982, 1990; Haslam & Wolseley, 1981 and Descy, 1976). Since the approach is relatively new, the sophistication of technique, such as is found in North American bioassays using invertebrates and diatoms, has not been developed in the European use of higher plants. The methods have been rather qualitative emphasizing indicator species. The process of developing macrophytes as a quantitative bioassay of stream water quality for eastern North America was initiated as part of the project described in this report. Comparisons of the higher plant communities of streams that are known to be pristine or high quality with those of similarly known degraded streams have been a key part of this effort. Once the groundwork has been laid and a baseline for comparison has been established, macrophyte monitoring of stream water quality is considerably faster and less expensive than current field bioassay techniques.

While invertebrates are very sensitive to heavy metal pollution and macrophytes are more tolerant, macrophytes are very sensitive to some pollutants which may not affect other aquatic communities except in higher concentrations (herbicides, for example) (Haslam, 1990). Changes in plant community caused by pollution will, in most cases, precede changes in the invertebrate community. These changes can be seen as early warning signs for the rest of the aquatic community and habitat. Perhaps the biggest advantage of macrophyte monitoring is that once the process is established it can be carried out by trained non-scientists and does not require the expertise of a PhD biologist or a highly-trained laboratory technician. Because macrophyte

monitoring is so quick, it can be used for large-scale surveys and to prioritize areas that need careful study by the costly methods of analytical chemistry.

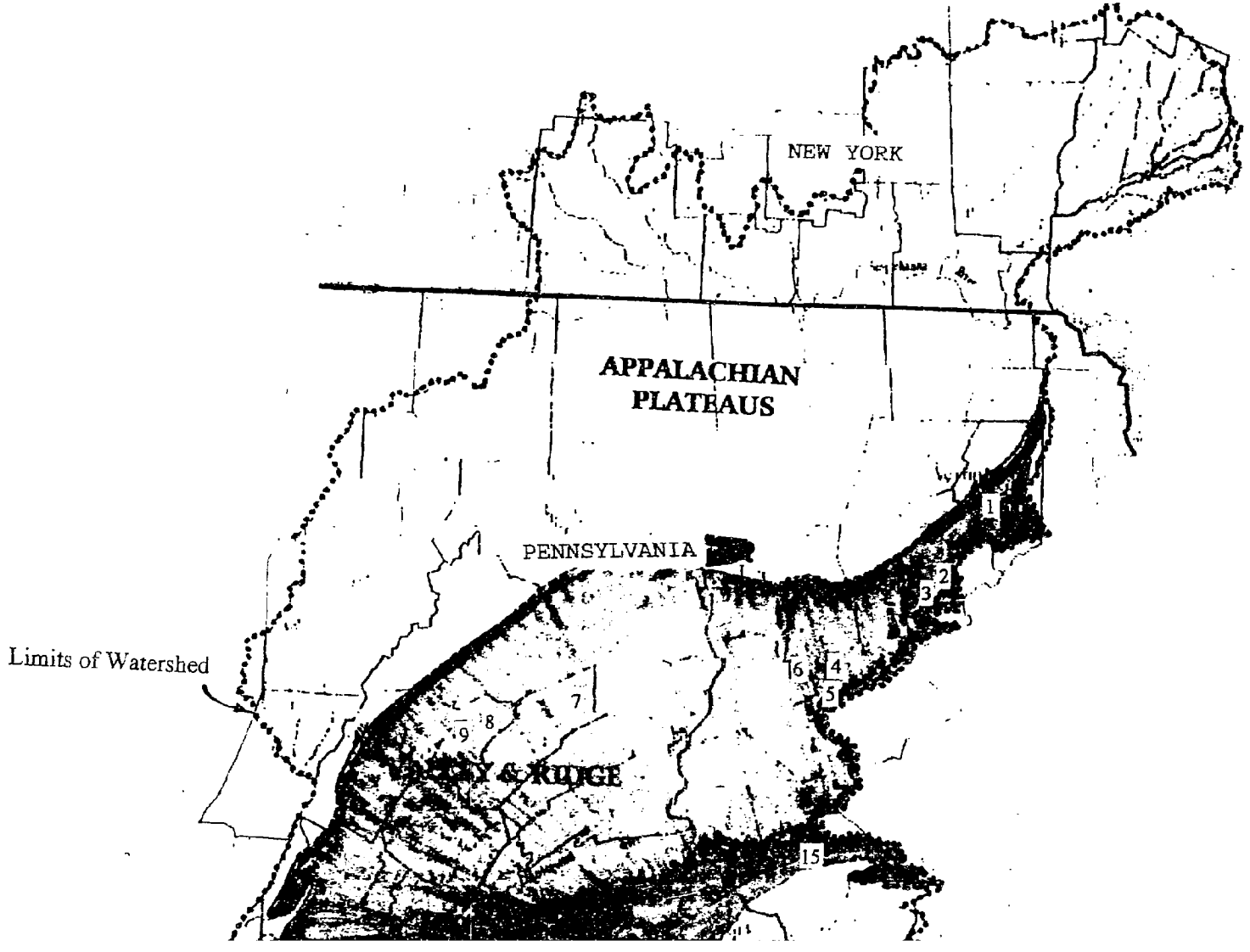
The macrophyte monitoring system detailed in these pages is still in the development phase. It differs from the European approaches in that it is both qualitative and quantitative, using index species and plant diversity techniques. Standardized sampling procedures and saturation curves for determining sampling levels are also employed. The data acquired by the techniques described in this report are amenable to the development of software that will allow the quantitative determination of the status of a stream in the field using a hand held calculator or a lap top computer. Usually, unless serious access or weather problems are encountered, a stream of moderate length can be surveyed by two trained technicians in less than one day. In many cases, the essential elements of the survey can be achieved in several hours.

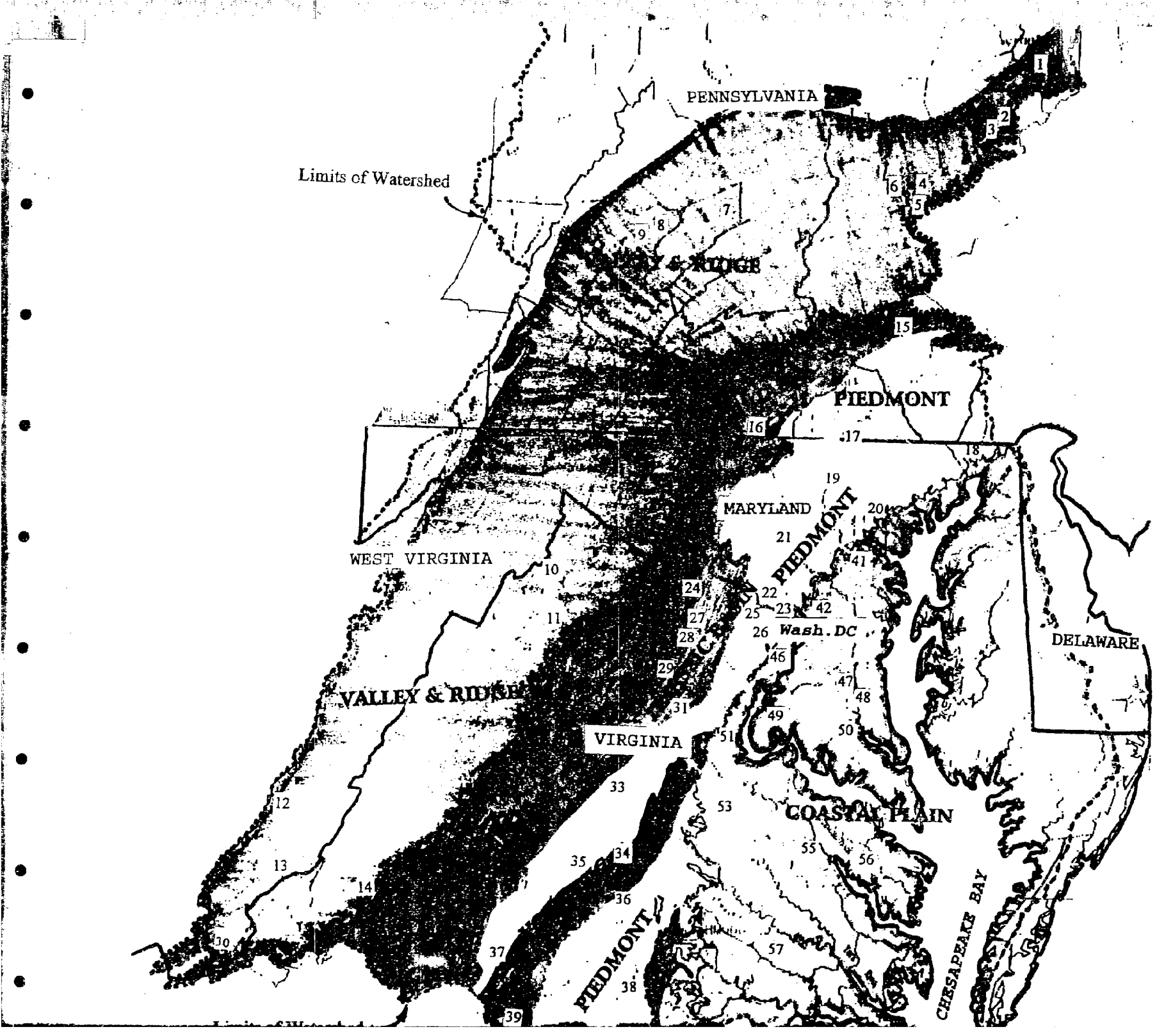
METHODS

Geomorphological Provinces

Using standard USGS topographic maps, 1:500,000 scale, overlain by a modified geomorphology of the bedrock geology as determined by the State Geological Maps (Virginia, West Virginia, Maryland, Delaware and Pennsylvania), a working wall-scale geomorphological map was constructed for the Chesapeake Bay Watershed (reduced version shown in figure 1).

MAJOR GEOLOGIC PROVINCES OF THE CHESAPEAKE BAY WATERSHED

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Limits of Watershed
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MARYLAND
PIEDMONT
WEST VIRGINIA
Wash. DC
DELAWARE
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COASTAL PLAIN
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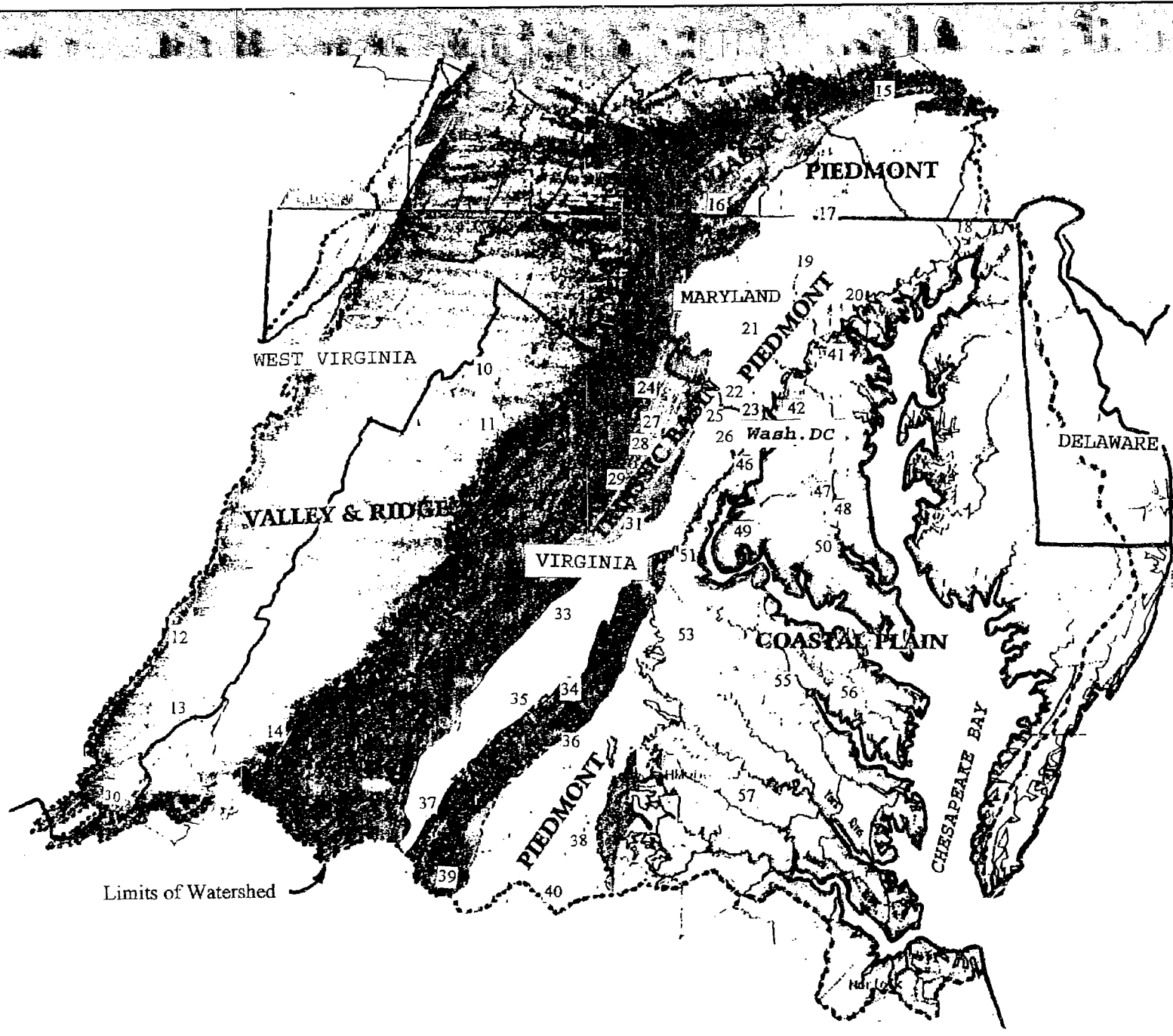

Figure 1. Major Geomorphological Provinces of the Chesapeake Bay Watershed as used in this study. Numbers designate "type" streams surveyed to date (see appendix A for key to stream names).

From this map, an appropriate distribution of reference streams for each geomorphological province was developed. (See appendix A. for number key to streams in figure 1).

At this time, baseline sampling of 60 streams has been completed for Coastal, Piedmont and Valley and Ridge Provinces of the Chesapeake Bay Watershed. Numerical analysis of 36 streams has been completed for Coastal and Piedmont Provinces and will be presented in this report. Initially the data of the Coastal and Piedmont Provinces is combined to show the general principles of macrophyte community structure and the basis of the macrophyte methodology. Then, the distinctiveness of the macrophyte populations of the Coastal and Piedmont Provinces is demonstrated. Finally, the manner by which macrophyte community structure and diversity can be used to determine stream water quality is shown.

Baseline Streams (Selection Criteria)

To prove the efficiency and the accuracy of the macrophyte bioassay techniques being developed in this project, reference streams of known water quality were necessarily included in the survey. The majority of the streams used for developing the reference or baseline data presented in this report were chosen from the most recently available and published state agency reports from Virginia, Maryland, and Pennsylvania (see "References" by state). The status of some Maryland streams was determined by contacting agency personnel directly involved in biological monitoring and collection of data used to compile these reports (personal communication, Niles Primrose, 1994). Initially, the biennial state reports required by the

Federal Water Pollution Control Act under section 305(b) were used and streams selected from these were cross checked against the other available publications.

The Maryland and Virginia State Agency 305(b) reports, and the Pennsylvania Code Title 25 Chapt.93 Water Quality Standards, which satisfy the 305(b) requirements, are compiled to assess compliance with the 1972 Clean Water Act (CWA) fishable and swimmable goals. These agency reports are a summary analysis of surface water quality based on monitoring data and/or evaluations. State evaluations are based on land use descriptions, point source discharge, non-point source pollution, fishery information, and data collected by citizens involvement groups such as The Izaac Walton League of America's "Save Our Streams" volunteer water quality monitoring program. Evaluations are based, as well, upon agency staff knowledge of specific streams and water bodies.

Monitoring data for the 305(b) reports primarily include ambient water quality monitoring (dissolved oxygen, temperature, and pH), as well as fecal coliform bacteria and toxic substance analysis of fish tissue, sediments and water column. Additionally, in some cases, monitoring of macroinvertebrate benthic organisms by the state agencies provides direct information on the health of the aquatic communities.

These reports summarize water quality, based on some or all of the previously mentioned methods, by giving each specific watershed, or section thereof (referred to as waterbody segment (VA), sub-basin (MD), stream (PA)) a rating. These are as follows for these specific states:

Maryland:

Excellent - Water quality supports all designated uses or meets water quality goals. Biological life is generally dominated by sensitive and intermediate benthic macroinvertebrate species. Pollution-tolerant species occur infrequently.

Good - Water quality generally supports designated uses or meets water quality goals. Pollution is minimal. Sensitive and intermediate benthic macroinvertebrate species are present only in moderate numbers. Pollution-tolerant species may be present in low numbers.

Fair - Water quality is characterized by intermittent severe degradation or by continued low level degradation. Waters are considered marginal with respect to designated uses or meeting water quality goals. Intermediate species are dominant while pollution-tolerant benthic macroinvertebrate species occur in moderate numbers; few, if any, sensitive species occur.

Poor - Water quality does not support designated uses or achieve water quality goals. Severe degradation is often experienced. Pollution-tolerant benthic macroinvertebrate species are dominant, if present at all. Only a few, if any, individuals from intermediate species occur. No sensitive species are present.

Virginia:

Fully supporting (Clean Water Act goals).

Partially supporting.

Non - supporting.

Pennsylvania:

The Pennsylvania Code was used for choosing high quality streams only. These streams were chosen from the Special Protection Category and their rating was:

HQ - High Quality Waters- A stream or watershed that has excellent quality waters and environmental or other features that require special water quality protection.

EV - Exceptional Value Waters- A stream or watershed that constitutes an outstanding national, State, regional local resource, such as waters of national, State or county parks or forests, or waters that are used as a source of unfiltered potable water supply, or waters that have been characterized by the Fish Commission as "Wilderness Trout Streams," and other waters of substantial recreational or ecological significance.

For Maryland and Virginia some specific streams or sections of streams within a particular sub-basin, were given an additional impairment rating based on benthic macroinvertebrate monitoring at specific bio-monitoring stations (BMS). The streams are rated as:

NI =non impaired,

MI =moderately impaired, or

VI =very impaired (which is equivalent to MD's SI =severely impaired).

These streams made up the initial list of streams to be considered for our baseline data.

Streams from this list were then further considered for the geomorphological province in which they were located: Piedmont (includes Triassic, Volcanic-Plutonic), Valley and Ridge and Coastal Provinces. An even, geographical distribution of streams, based on the size of each zone, was considered important to the study. The lower Coastal zone (less than an elevation of 25 feet along the Chesapeake Bay) was not included within this phase of our research. This is due to the large percentage of marsh and swamp areas that cannot be rapidly surveyed without some modifications to our current technique. The goal was to survey as many streams in as many geomorphologic zones as possible within a growing season.

When a list of candidate streams within a geologic zone was compiled, along with their BMS locations, 7.5 minute quadrangle topographical maps from the U.S. Geological Survey were obtained for these streams. These topographic maps were also used to determine access points at regularly spaced intervals along the portion of the stream's length which was considered for survey. The age of the data used to make these maps (most were compiled at least 10-15 years ago), make them difficult to rely on. This necessitated scouting trips to each stream before the actual survey. The maps were then taken into the field for current evaluation of recent land use changes. During these scouting trips, a stream's accessibility was confirmed or rejected.

Accessibility was gauged by a stream's conduciveness to a complete survey consisting of 20 sites in no less than 5 miles.

Scouting of streams from the initial list, which contained specific BMS data, and removing from the list those candidate streams not fitting our criteria for accessibility, left us with a shortage of streams to survey in some geomorphological provinces. Returning to the state agency publications, we then selected additional streams to fill out a geologic zone based only on the stream's presence within a sub-basin of known quality (MD), CWA compliance (VA), or usage (PA). These streams were then scouted for accessibility and occasionally our initial criterion of at least five miles for a complete survey of 20 sites was altered to at least two miles for 20 sites.

Field Methodology and Data Collection

A stream considered for assessment was first scouted for feasible access points. The topographical maps were checked for road bridges, property bordering the stream, and jeep or walking trails running close to or crossing the stream. These points were then checked first-hand to determine their accessibility.

Access points were chosen based on the following criteria:

1. Width and depth of the stream - the stream had to be shallow enough to walk across or narrow enough to see the plants on the opposite bank.

2. Stream characteristics - the stream had to be flowing and not exhibiting marsh or swamp characteristics.

3. Private property - if the stream flowed through private property on which a "no trespassing" sign was present, permission was obtained from the property owner before surveying that section of the stream.

Once the access points for a stream were chosen, the number of sites were distributed equally between them, with an attempt to achieve as close to 20 sites per stream as possible. The sites at each access were approximately 60 to 90 meters apart, and at least 30 feet from a disturbed access point (i.e., bridge or culvert). To estimate the distance between sites, either pacing (counting previously measured steps), a pedometer, or range finder was used. Sites were numbered from upstream to downstream.

Before recording any data, the site transect was defined. Transects were 15 feet long and included both banks. The banks were specified to include all vegetation growing either within three feet of the water's edge, or from the water's edge to the top of the bank, whichever was shortest.

After the site location and boundaries were determined, the parameter data was recorded onto pre-made data sheets. The following parameters were noted:

1. Width: the approximate width of the stream, from water's edge to water's edge at the widest point within the site boundaries, was roughly estimated by sight. For both this parameter

and the next (depth), as well as general working efficiency and safety, field work was not undertaken during or after heavy rains.

2. Depth: the approximate depth of the deepest point within the site boundaries was roughly estimated by sight.

3. Substrate: the following definitions were used to determine the substrate:

boulders - stones requiring more than one hand to pick up (due to size - can be huge).

rocks - stones that can be picked up with one hand.

gravel - stones that can be picked up in the fingers and that have a rough surface.

sand - small enough grain size to fit on the tip of the finger.

mud - fairly solid, wet, sticky, soft earth.

loam - mix of sand, clay, silt and organic matter.

muck - extremely soft earth that does not support weight.

clay - firm earth that has plasticity when wet.

silt - top layer of fine sediment on the stream bed bottom.

bedrock - solid rock comprising stream bed.

4. Bank heights: facing downstream, the left bank is on the left side. The height can be estimated by sight from the water's edge to the top of the banks (regardless of the site boundaries).

5. Percent canopy cover: determined by using a spherical densiometer (from the Ben Meadows Company). The spherical densiometer takes into consideration canopy cover at all times of the day. The measurement was taken from the middle of the transect whenever possible.

In addition to these parameters, each plant species and its abundance was recorded for each site onto separate data sheets. Plant abundance is an estimate of the number of individuals of each species (i.e., plants fell mostly, but not always, into the categories 1, 5, 25, 50, 100, or 1000). For certain species (such as Fontinalis novae-angliae) a less specific estimating method was necessary, as is very difficult to determine where one plant ends and the next begins. In these cases, a specific size area was assigned for each species to equal one plant (i.e., 1 Fontinalis sp. = 10 x 10 cm square). Likewise, problems occurred with species that have an inconspicuous rhizome. For these species, each apparent plant was counted as one, whether or not it was part of a larger plant (this was to reduce confusion and error when the study is performed by non-experienced surveyors).

Specific categories of plants were excluded when conducting field surveys of the Piedmont and Coastal Plain Provinces. These categories are:

Woody plants: mostly trees and shrubs that remain from season to season are excluded because they are less likely to show the more immediate responses to pollution than are the more seasonal herbaceous plants. They were, however, retained in Valley and Ridge Province due to the relatively small number of species in this zone.

Vines and brambles: due to the difficulty in determining if their point of origination lies inside or outside an actual transect site. (Retained in Valley and Ridge Province).

Grasses: due to the difficulties inherent in identification of species.

Taxonomy

The majority of plants were easily identified at each field site, using standard taxonomic keys, by the general biologists (not trained botanists) that constituted the field teams. Plant individuals that were not easily identified in the field were collected in sample bags, and provided with a numerical designation that corresponded to the stream and site of the collection. These plants were also photographed for identification by a trained botanist in the laboratory. After one season of training, all species used in the analysis could be identified by the field team. Volunteers with no biological training can be quickly taught to identify the eleven key indicator species.

The plants collected at each site were pressed as soon as possible to preserve their quality. Each unknown specimen was labeled with its nickname, stream of origin, geologic zone, sample bag number, photo number and site number. The plants were later identified by a professional botanist contracted by the Marine Systems Laboratory and given access to the Natural History Museum's Department of Botany Collections. Note that this process was carried out only in this phase of research and development and will not be part of the final methodology. As we discuss

in depth below, macrophyte species that are often difficult to identify are not included in the bioassay technique being developed.

Approximately 800 plant specimens were collected and eventually identified. Identification yielded a total of 308 different species. Keys and identification guides consulted are listed in the "References" section of this report. A procedures and identification manual is under development and was presented, in preliminary form, in the interim report dated Feb. 1994. We have not included that manual in this report.

Analysis

Prior to statistical analysis and removal of problem species from the master lists, each species found in the field studies was given a weighting factor. This weighting emphasized the importance of the aquatic environment to that species and therefore to stream quality determination. The weighting factors are based on the wetland relationships as assigned by the Fish and Wildlife Service. The categories were obtained from the National Lists of Plant Species that Occur in Wetlands: Northeast (Region 1), Biological Report, 88 (26.1).

The wetland relationships assigned by the Fish and Wildlife Service along with the estimated percent probability for a species in that category to occur under natural conditions in wetlands are given below. The weight factor that corresponds to each is as follows:

Weight Factor	Indicator Categories
2.0	Obligate Wetland: >99%
1.5	Facultative Wetland: 67-99%
1.0	Facultative: 34-66%
0.5	Facultative Upland: 1-33%
0.5	Obligate Upland: <1%

When different species within a genus were impossible to differentiate in the field, they were combined as "Genus sp."(i.e., Callitriche heterophylla and Callitriche deflexa were combined as Callitriche sp.). If they all had the same weight, this was the value assigned to the combination. However, if the species had different weights, the combination was given a median weight of 1.0. Six of the 198 taxa used in the general list fall into this category.

Once all the data had been collected from the field, composite "master" lists were made of all species seen in each geomorphologic province with their corresponding weight factors. For both the Coastal Plain and Piedmont Provinces treated in this report, the master list included 198 species, and from this list community structure and indicator species analyses were performed.

Prior to statistical analysis, the master list was reduced to eliminate rare species or those considered difficult to identify in the field. This elimination of species was necessary to reduce

the amount of time spent in the field for the final methodology. Several factors were taken into consideration in determining which species should be eliminated for diversity. Primarily, if a species occurred in more than one third of the high quality streams within a geomorphologic province, it was retained for that zone. If a species occurred less frequently, its weight factor, abundance and degree of difficulty of identification were then considered. This left the reduced master list with 61 species for the Coastal Plain Province, and 89 for the Piedmont Province.

A series of programs was written within the Statistical Analysis Software System (SAS) with the aid of statisticians and SAS experts from the Smithsonian's Office of Information Resource Management. The reduced master list was used for the programs created to: 1) determine the minimum number of sites required to achieve a saturation of species number on any stream; and 2) to calculate and plot several diversity indices.

To include parameters of local stream variability and random or determined access characteristics, it was important to sample each stream until the number of macrophyte species that characterized that stream were located. Thus, from the data set for each stream (derived from the reduced master list) a "saturation curve" was developed by taking every possible combination of station order and determining the summed number of total species found after one, two, three, etc. stations. This was accomplished in the laboratory, but it can also be achieved in the field with a lap top computer or a programmable calculator. Typically saturation

on low quality streams was achieved by about 15- 20 stations. High quality streams were often not saturated by 19-20 stations. This issue is discussed further in the "Results" section.

RESULTS

Macrophyte Community Structure

In figures 2 and 3, the randomized mean species saturation curves for the Piedmont Geomorphological Province and the Coastal Geomorphological Province are plotted separately as high and low quality streams. Also shown are the standard deviation and maximum and minimum values at each number of stations. Even with as simple a measure as a defined frame species number, most high quality and low quality streams separate at a relatively few stations. Also, clearly Piedmont streams have higher species numbers, on the average, than Coastal streams.

For the Piedmont Province, high and low quality streams can be separated at five or more stations with a confidence level of $p \leq 0.0001$ (using both the Cochran and Cox and Satterthwaite methods of t-testing). Significance increases as the number of sites increases. Because of the apparent lower quality of high quality Coastal streams (see below), the mean difference between high and low quality Coastal streams is less significant. Using the Cochran and Cox method, significance is achieved by 19-20 sites, with the lower sites rising to $p=0.06$. All of the Coastal sites show a significant difference by the Satterthwaite method of t-testing.

PIEDMONT PROVINCE

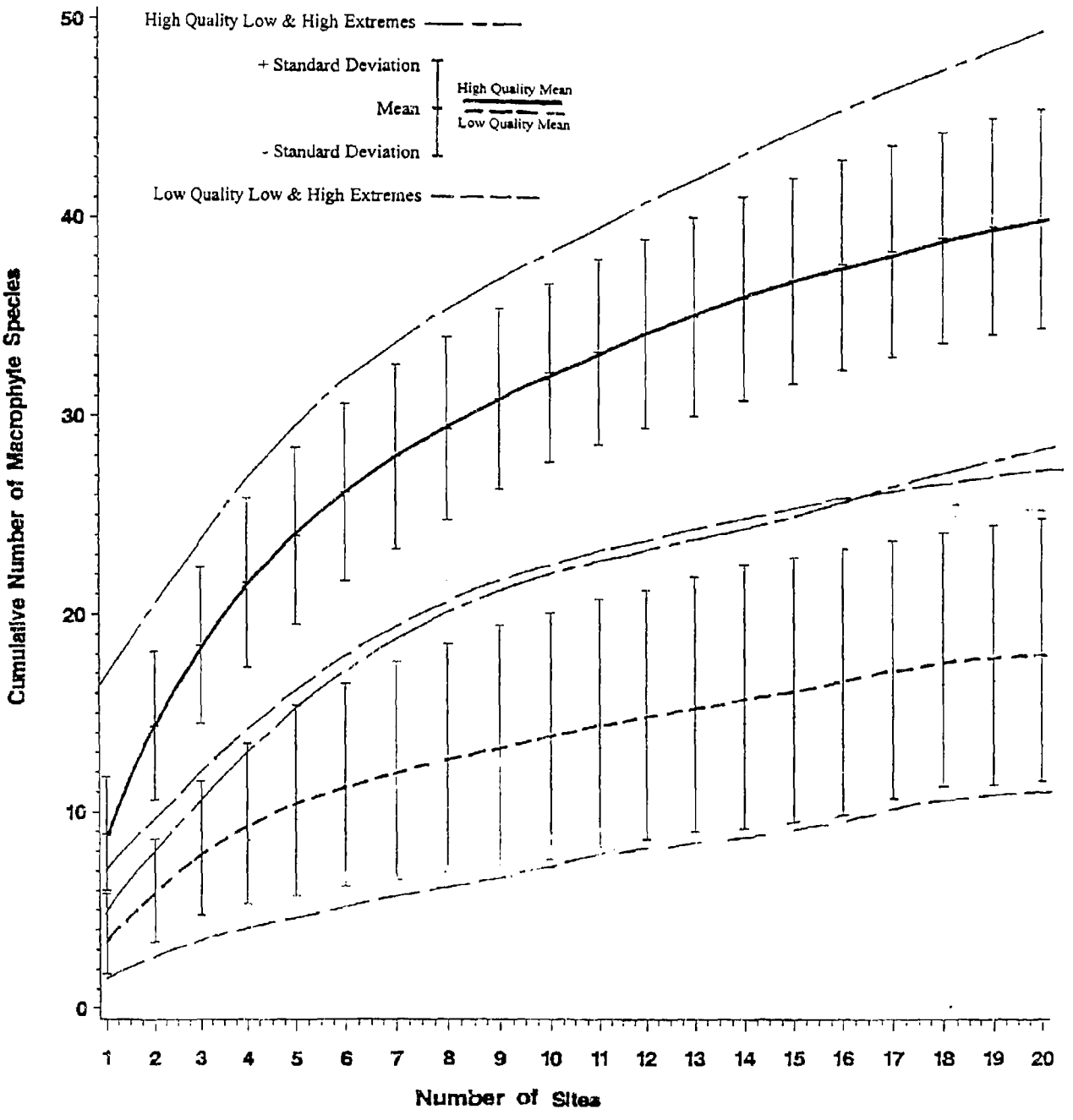

Figure2. Mean, standard deviation and range of defined-frame macrophyte species tallied by summing every possible order of station occurance for all streams.

COASTAL PLAIN PROVINCE

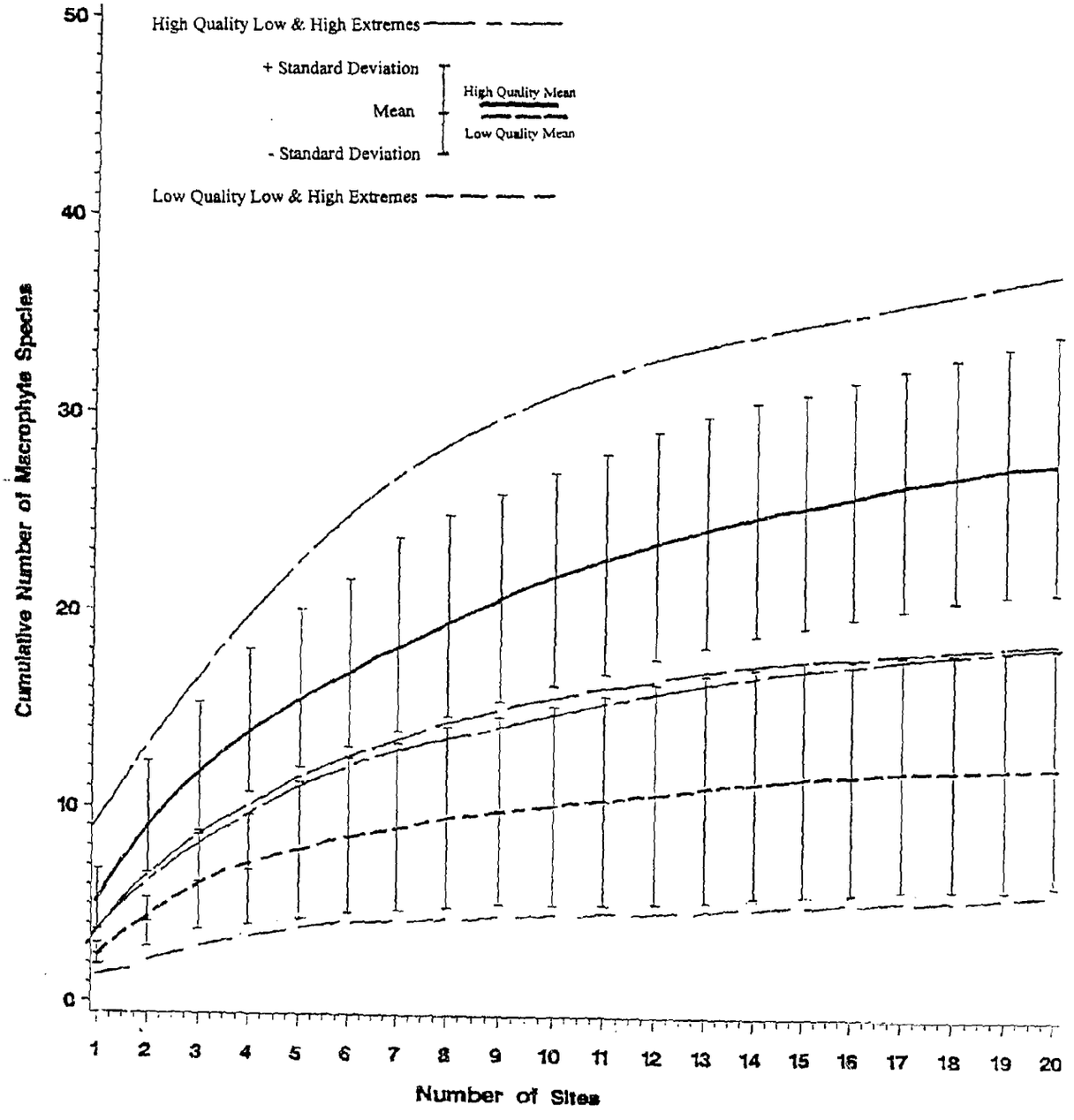

Figure3. Mean, standard deviation and range of defined-frame macrophyte species tallied by summing every possible order of station occurance for all streams.

For the entire Coastal - Piedmont macrophyte reference stream data set described above, a plot of numbers of species as a function of the numbers of individuals of those species is given in figure 4. The data taken from high quality and low quality streams are plotted separately and the interval used is a doubling factor or series of octaves plotted in the form of the standard Preston curve (1948). This " log normal" curve demonstrates that numerically the community structure for macrophytes, regardless of stream quality, is quite similar to that obtained for many taxonomic groups from other environments. However, not only do the low quality streams of the entire data set have significantly fewer species than high quality streams, but the numbers of individuals of the entire community are also significantly reduced.

For each high and low quality stream of the entire Coastal and Piedmont data set, the mean number of macrophyte individuals per stream is plotted as a function of total macrophyte species number (Fig. 5). This plot directly demonstrates that both the number of species and the number of individuals is greatly reduced in low quality streams (on the average to 45% of the species and 32% of the individuals). Although there is relatively little high quality / low quality overlap for this data set, it is clear that the variability in the macrophyte community of high quality streams, as defined by State surveys, is considerably greater than that in low quality streams. The differences between high and low quality streams for species/plant numbers are highly significant, by a Multivariate Analysis of Variance for combined parameters ($P=0.0001$).

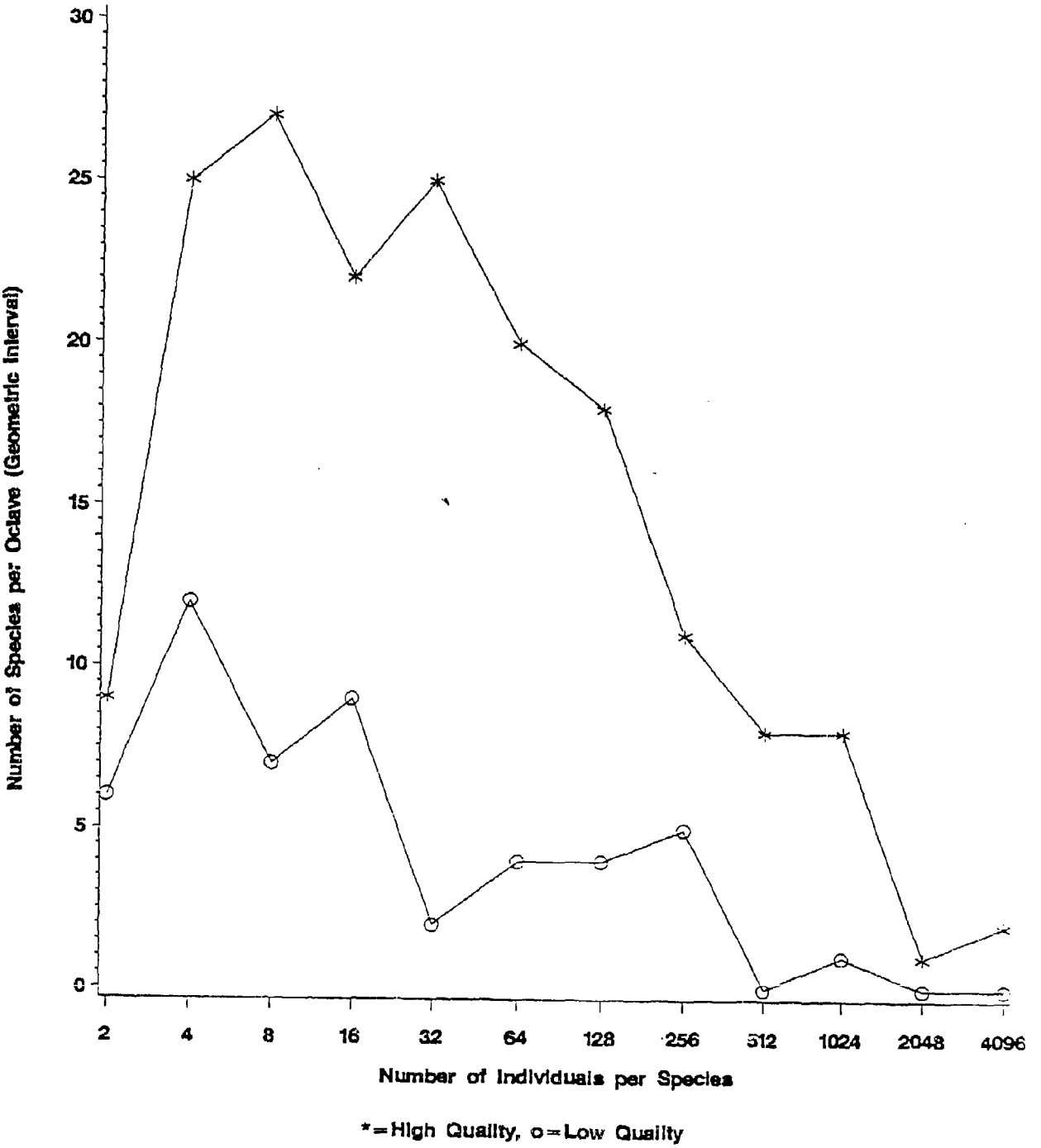

Figure 4. Number of species as a function of the numbers of individuals of those species.

Comparison of HIGH and LOW Quality Streams

Number of Plants Vs. Number of Species for Each Stream

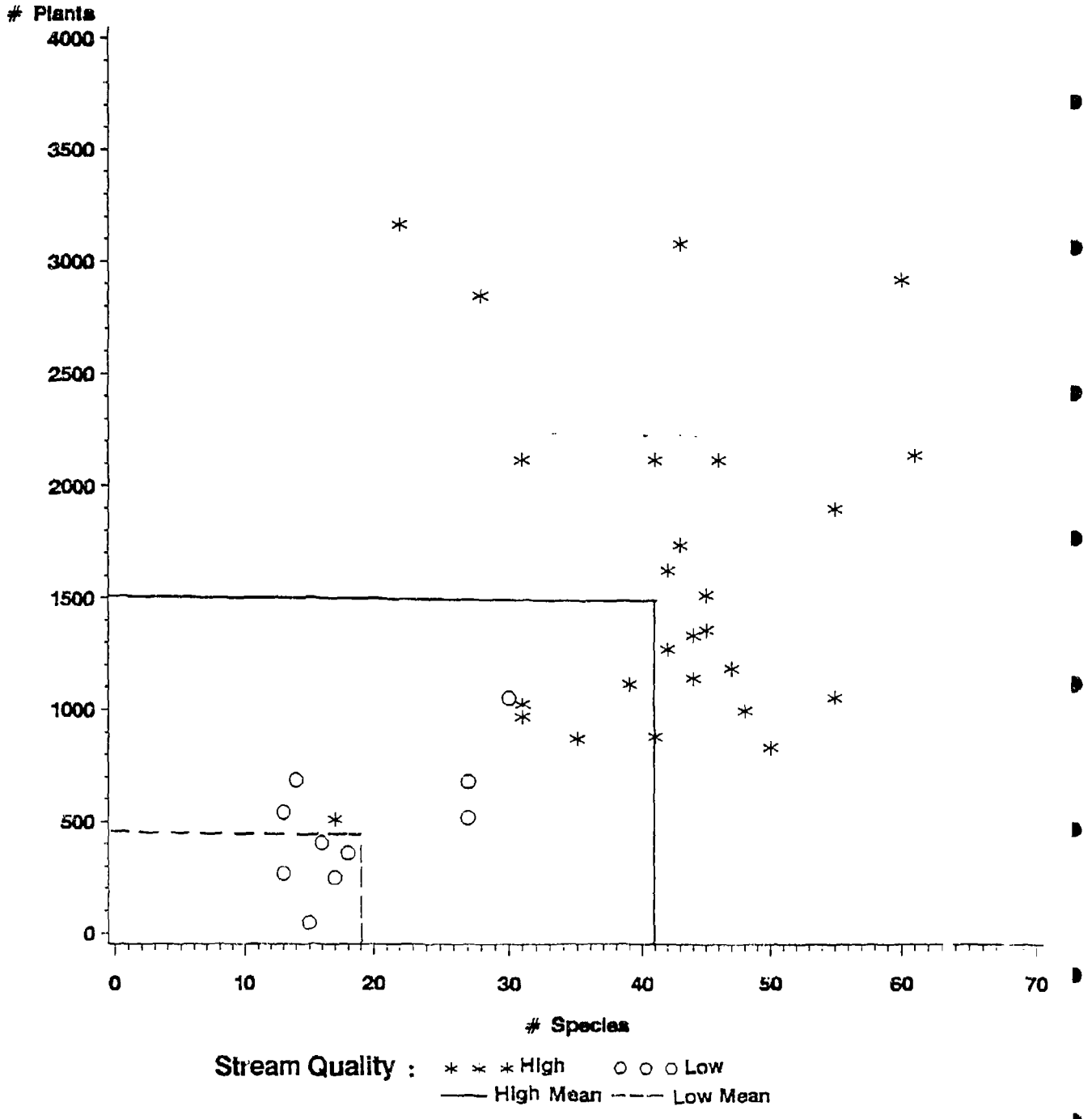

Figure 5. Number of plants (individuals) of all species for each stream of the Piedmont and Coastal data set. Values are means of all stations within a stream.

Using the same data set as in figure 5, figures 6 and 7 are separate plots, each comparing the Piedmont and Coastal Geomorphological Provinces. High quality streams from the Piedmont Province have a significantly larger mean number of species (at the 0.0001 level) than those of the Coastal Province. The numbers of individuals are not significantly different. The low quality streams from the two provinces are not significantly different in either regard. Because high quality streams of the Piedmont have about 50% higher species number than the high quality streams of the Coastal Plain, a greater differentiation capability can be achieved by treating them separately, although the respective data sets are smaller.

Indicator Species

To examine macrophyte community structure at the species rather than the stream level, the mean number of individuals of each species for all streams has been plotted as a function of percent occurrence of each species in all streams. These data are shown, separated by their respective values in high and low quality streams, in figure 8. The complete list with species names and coordinates on the figure appears in appendix B. Exponential curves fit by least mean squares, fit separately for both high quality and low quality streams, again clearly demonstrate the differences in their macrophyte community structure status. The curves are significantly different as shown by an F-test at P=0.01. It is apparent from the figure that for both low and high quality species there is a large quantity of rare species with low numbers. It is particularly interesting, however, that four macrophyte species of high frequency for high quality streams (>90%) show a considerable drop in the number of individuals, and in some cases the

Comparison of HIGH and LOW Quality Streams

Number of Plants Vs. Number of Species for Each Stream
QUALITY = High

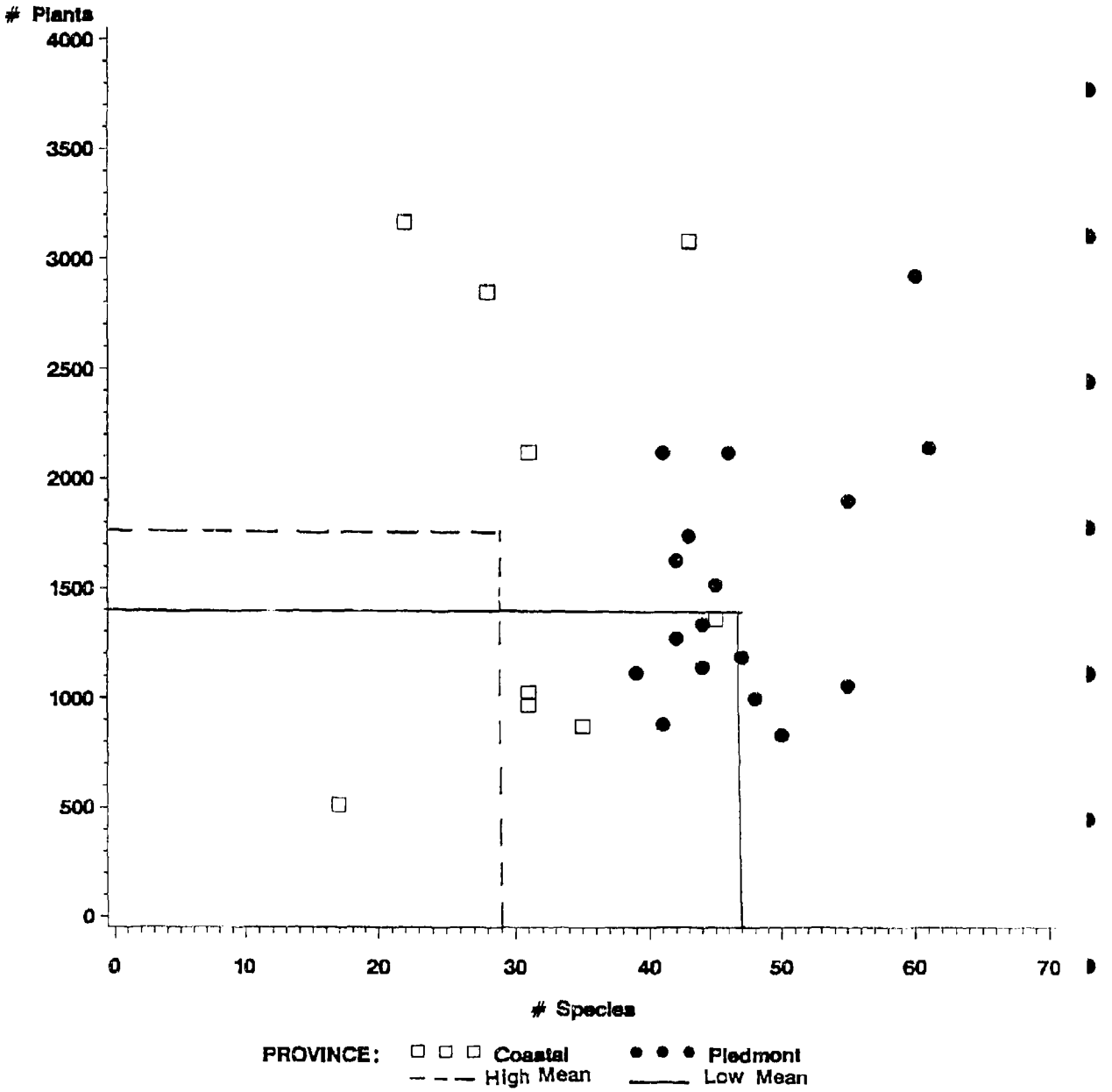

Figure 6. Number of plants (individuals)of all species as a function of the number of species for each high quality stream.

Comparison of HIGH and LOW Quality Streams

Number of Plants Vs. Number of Species for Each Stream
QUALITY = Low

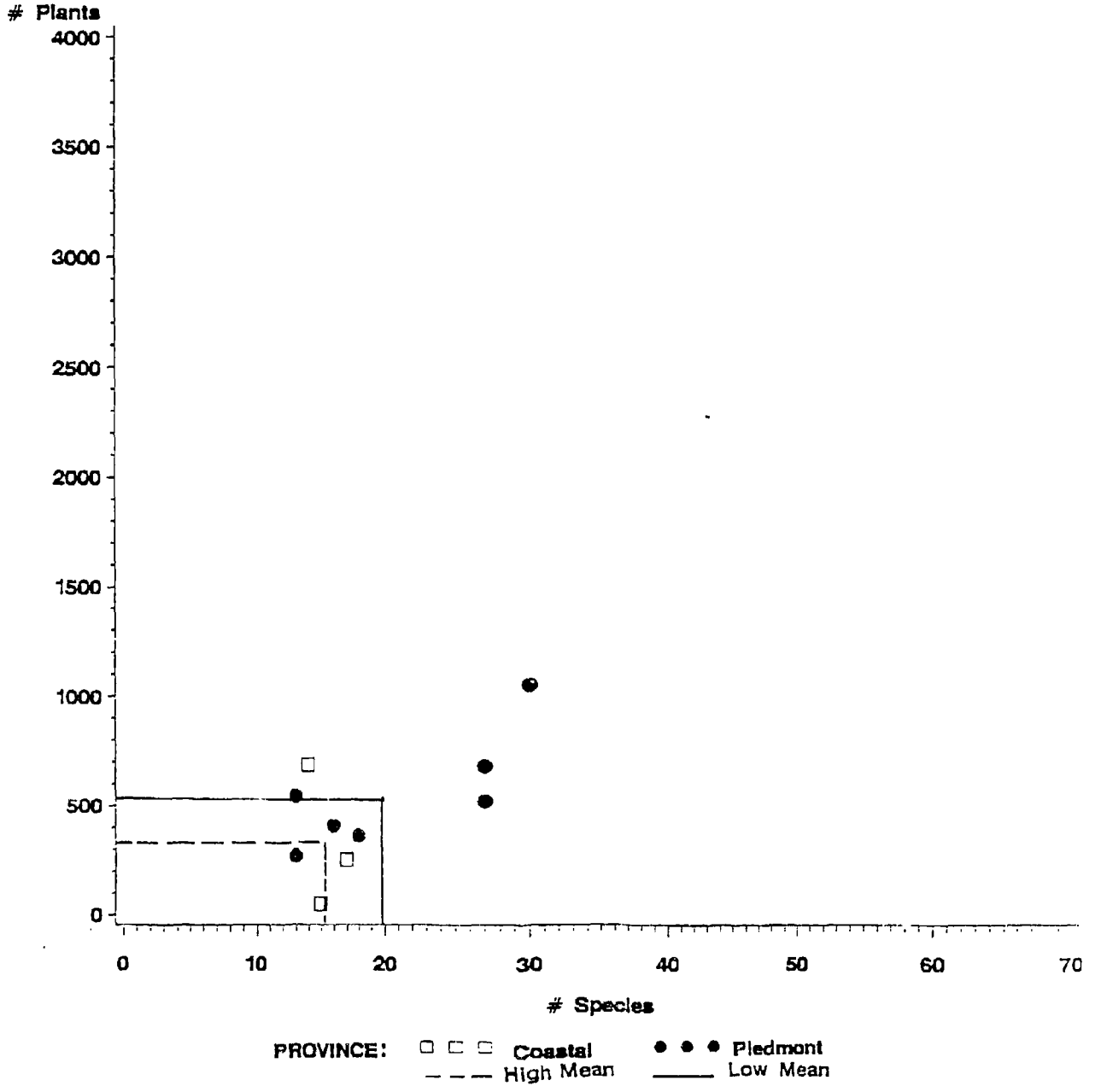

Figure 7. Number of plants (individuals)of all species as a function of the number of species for each low quality stream.

Comparison of HIGH and LOW Quality Streams

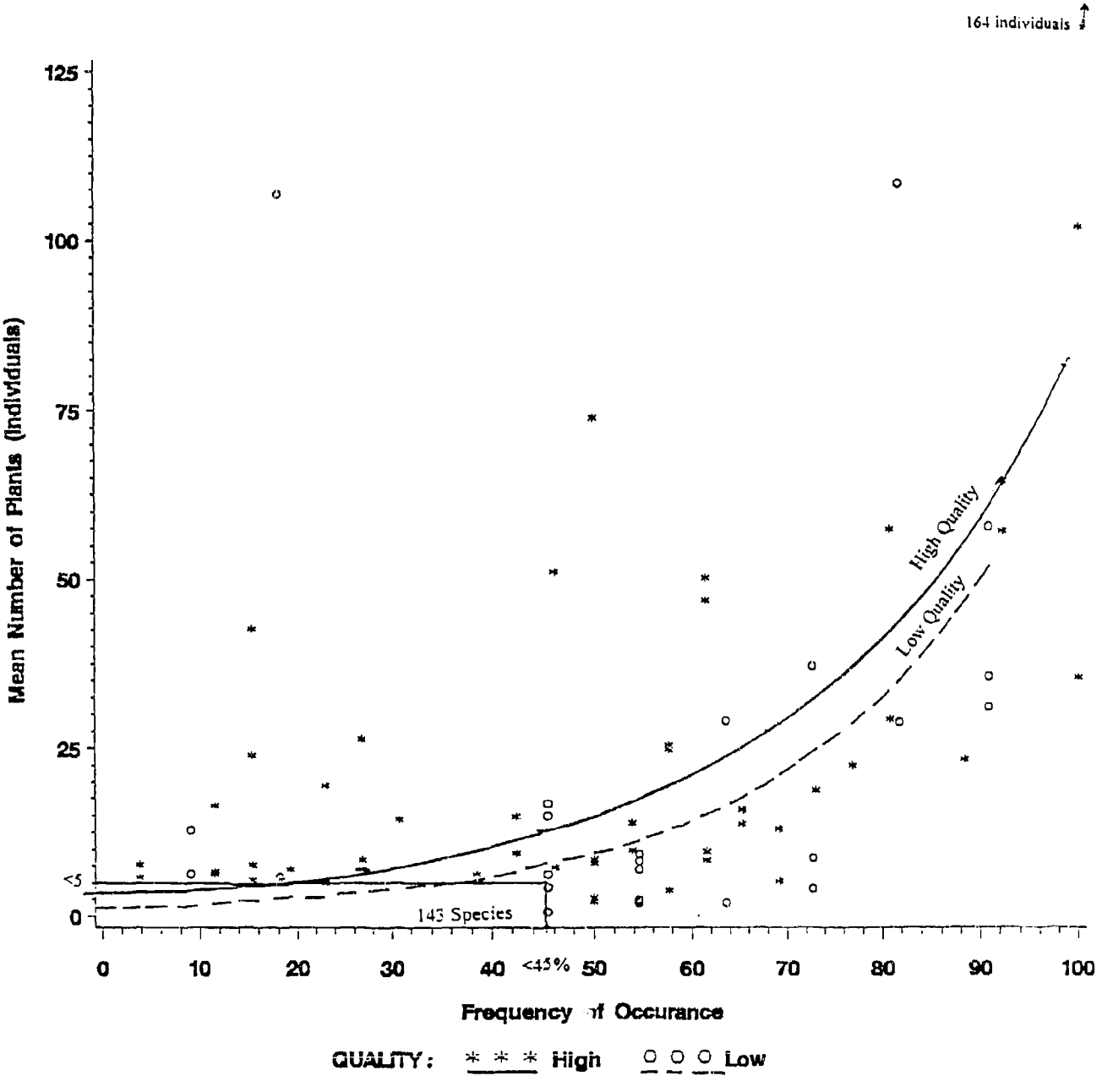

Figure 8. Mean number of plants (individuals) of each species as a function of the frequency of occurance of each species. Values of each species are plotted separately for high quality and low quality streams. In the lower left block where individual data points are not shown, 143 species have both high quality and low quality points within this block.

frequency of their occurrence, between high and low quality (see figure 9). Since these plants are potentially ideal indicator species, the mean combined numbers of individuals for these four species are plotted in figure 10 by stream and stream quality status. The difference between the means is significant by the correspondence analysis procedure (P= 0.05). The single low quality stream that lies well out in the high quality field is South Run of Fauquier County, Virginia, a stream of only moderate impairment on the State of Virginia scale. Nevertheless, there is more overlap of high and low quality streams here than in the direct plot of the number of individuals as a function of species number (Fig.5).

Another series of potential indicator species is also shown in figure 9. These are species of moderate numbers of individuals that drop considerably in frequency (i.e., the number of streams in which they occur) from high to low quality. Seven species have been selected as potential indicators and plotted as separate high and low quality sets in figure 11. As with the indicator species selected for loss of individuals, the difference between the two means is highly significant (P=0.005). However, again there are many streams in the overlap zone providing only weak discrimination as a test for individual water quality.

The indicator species analysis presented above has shown the same features of strong overlap of streams of different qualities that have generally characterized the so-called "qualitative" analyses of invertebrates and diatoms. On the other hand, if both indicator species sets are plotted against each other for each geomorphological province (Figures 12 and 13), a considerably greater level of differentiation is derived.

Comparison of HIGH and LOW Quality Streams

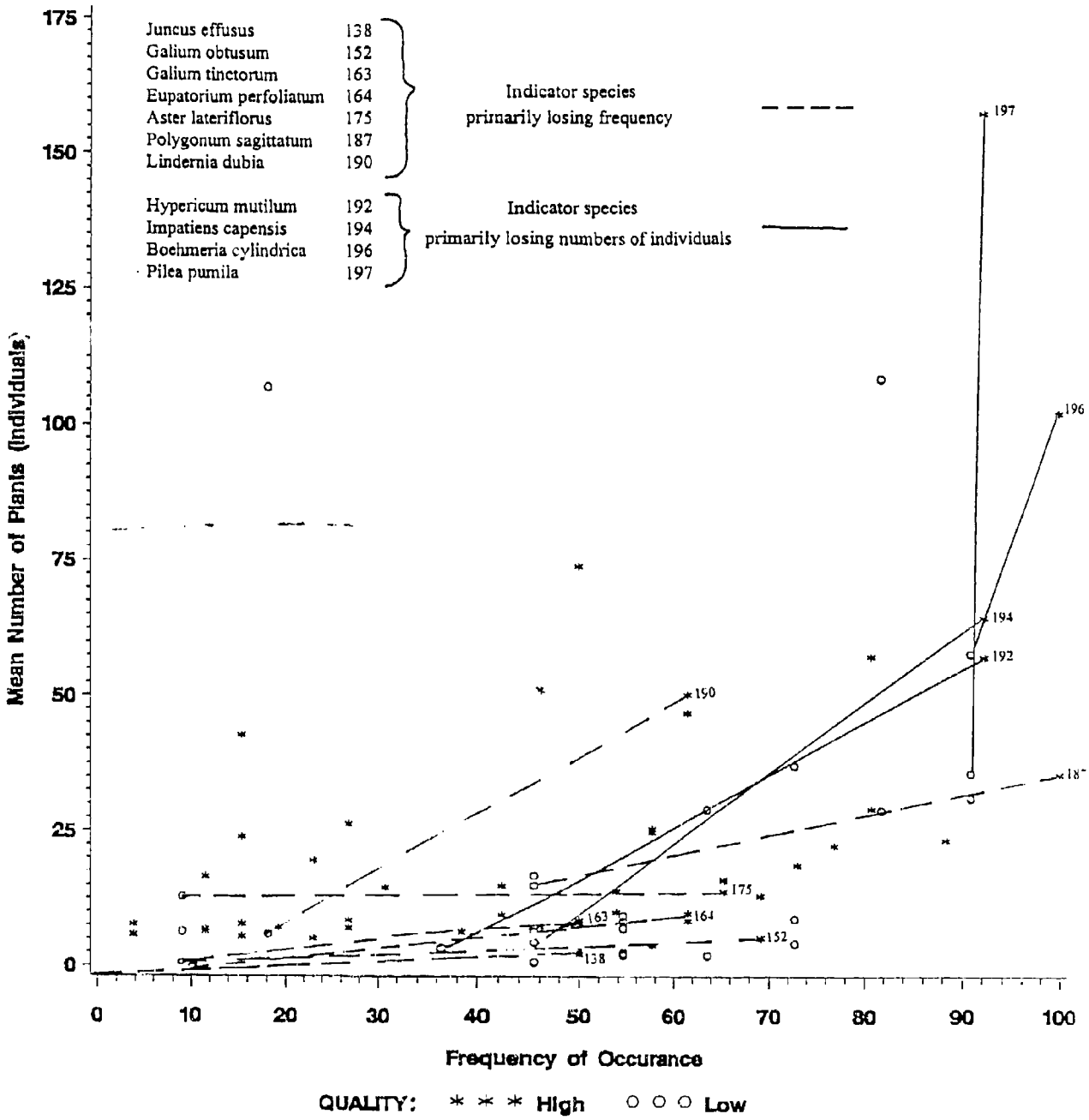

Figure 9. Change of frequency and abundance of indicator species shown on framework of Figure 8.

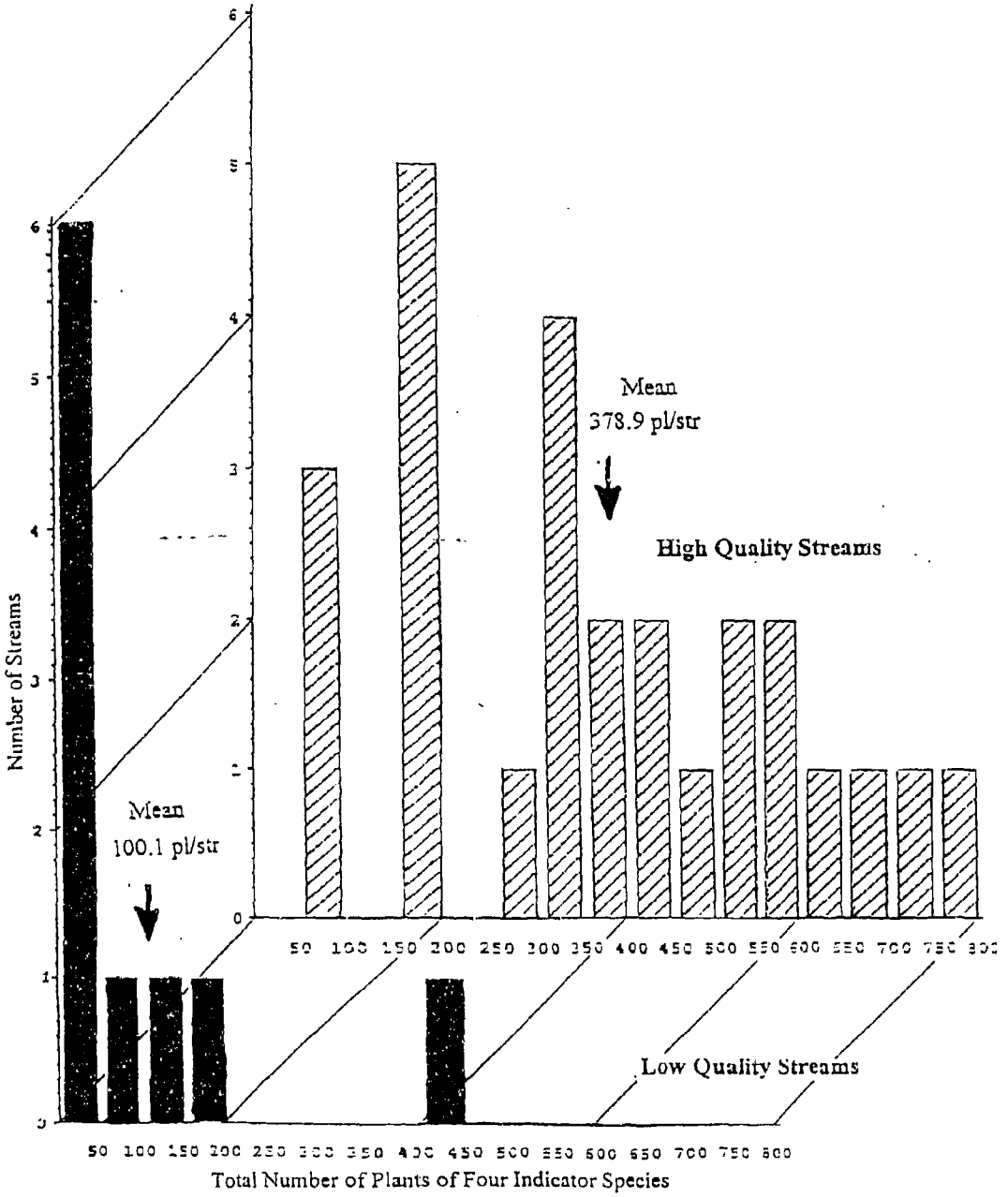

Figure 10. Change in status of four indicator species that tend to lose numbers of individuals with drop in water quality. Streams of both Piedmont and Coastal Provinces.

Distribution of Streams by Loss of Indicator Species

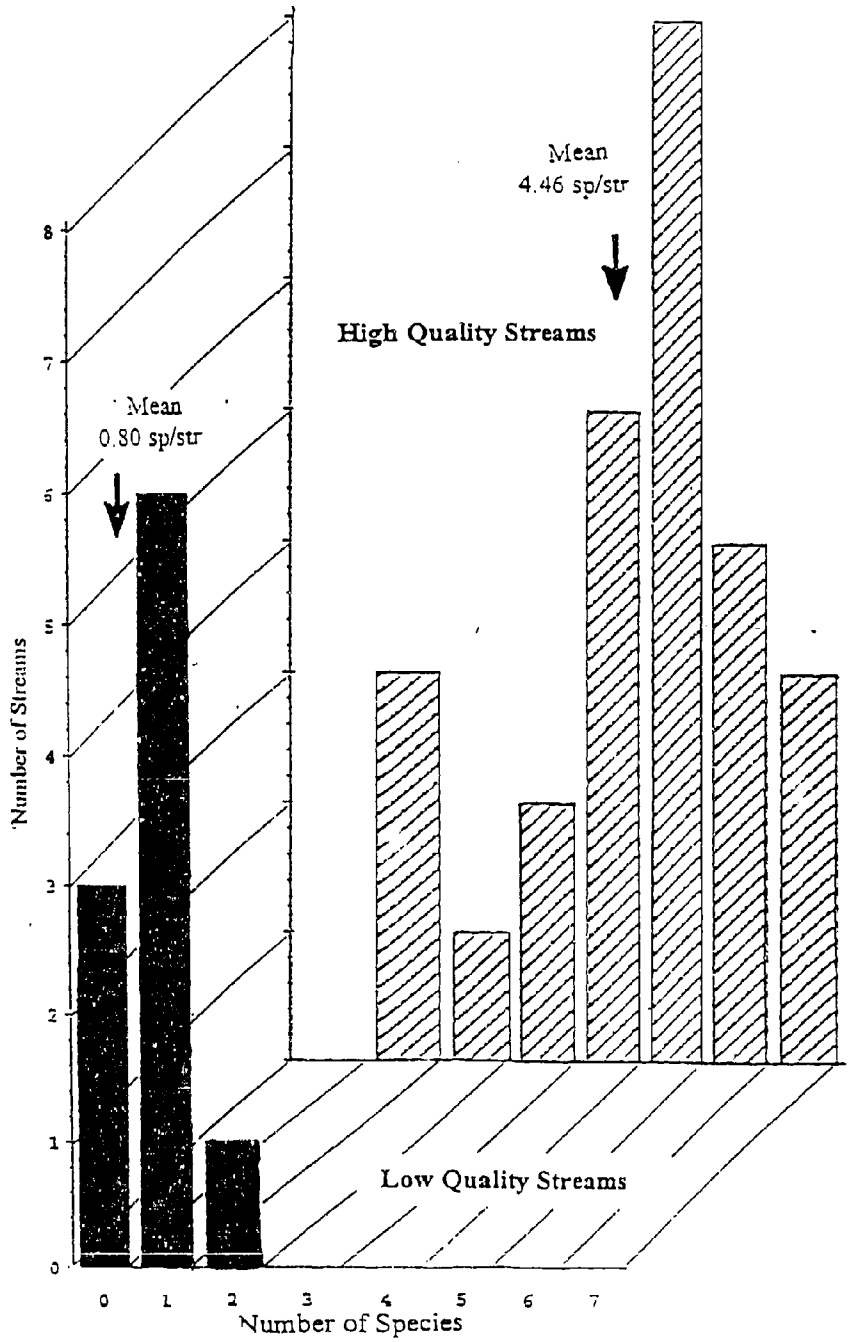

Figure 11. Change in status of seven indicator species that tend to be lost with drop in water quality. Streams of both Piedmont and coastal Provinces.

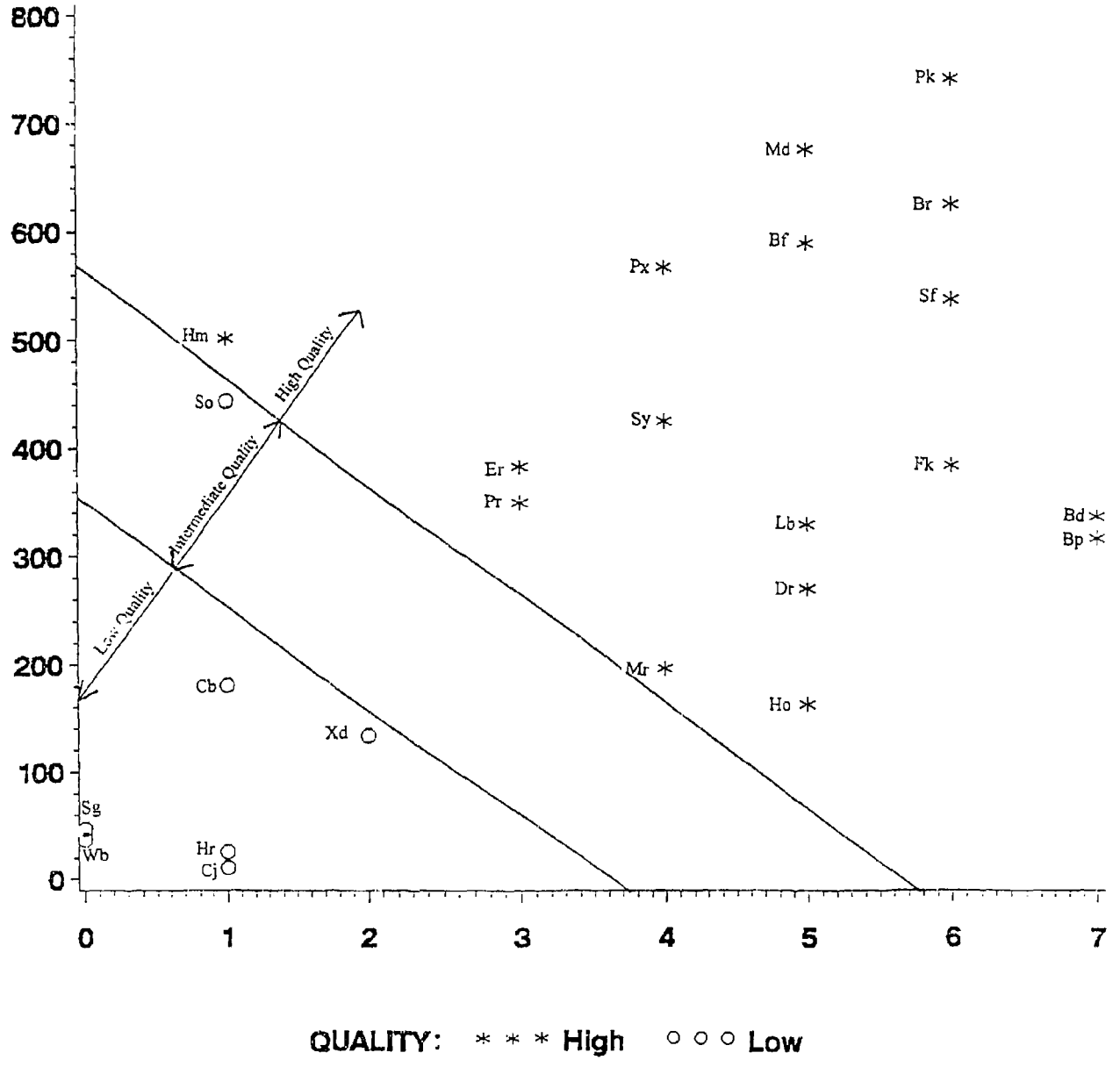

Figure 12. Number of plants (individuals) four indicator species that tend to lose numbers (Figures 9, 10) as a function of seven indicator species that tend to be lost with reduction in water quality. Piedmont Province. For stream designations, see Appendix A.

Distribution of Streams by Two Sets of Indicator Species

PROVINCE = COASTAL

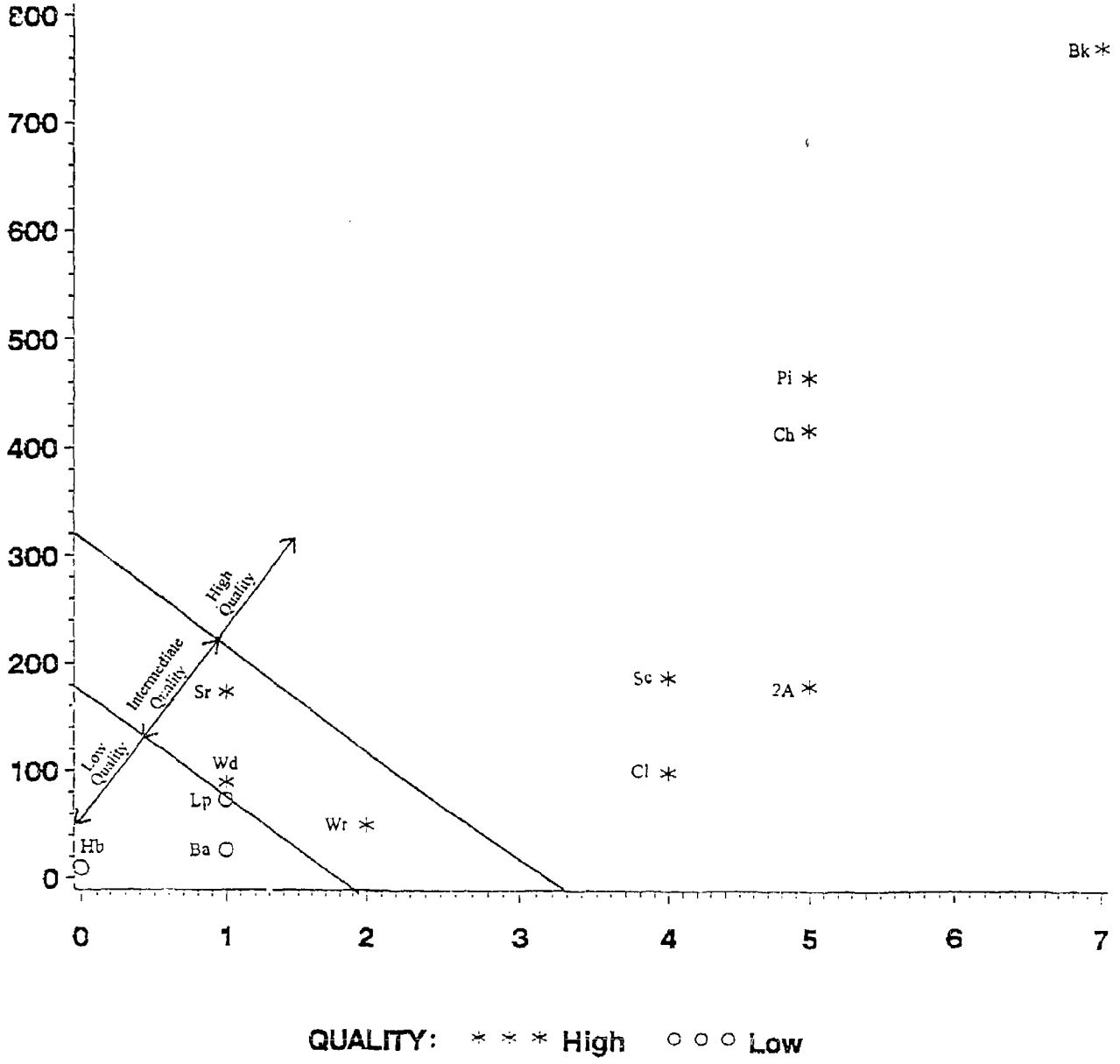

Figure 13. Number of plants (individuals) of four indicator species that tend to lose numbers (Figures 9, 10) as a function of seven indicator species that tend to be lost with reduction in water quality. Coastal Province. For stream designations, see Appendix A.

The Piedmont Province shows only a single "low quality" stream of near overlap, South Run, which is characterized as only moderately impaired by the Virginia Water Quality Control Board. The Coastal Province shows no overlap between high quality and low quality streams. However, there are three "high quality" streams grouped close to the low quality field. These streams also fall well into values for low quality streams in the Piedmont. We have chosen to designate these as intermediate in quality, recognizing that this is more likely a definitional problem rather than an issue of discriminational capability.

Drawings of all eleven indicator species are shown in appendices C and D.

Diversity Indices

To avoid the problems of using single species, or a narrow array of species, as indicators of water quality degradation, species diversity indices have been widely used as a measure of whole ecosystem function. However, rarely has species diversity in this context represented total biodiversity. Rather, for practical reasons, considerably more limited groupings or taxa, e.g., insects, diatoms, etc., have been employed. Here, the same basic approach as was applied to aquatic macrophytes is presented. In this investigation, the spectrum of macrophytes to be considered has been limited to 61 species for the Coastal Province and 89 species for the Piedmont Province, by dropping off rare species and difficult-to-identify species. The purpose for this is to truly render a rapid analysis that is achievable by field workers with a moderate level of training. Inclusion of rare and difficult-to-identify species would improve the discrimination capabilities of the technique. However, discrimination of streams into water

quality categories appears to be quite adequate and it is difficult to justify the inevitable increase in time and cost.

Unlike the situation in generally open landscapes, in much of eastern North America a stream's macrophyte diversity is sometimes a function of tree cover. Thus, where a full range of cover is available, in figures 14 and 15, we have plotted species diversity as a function of cover. Where only high cover stations are available (above 66% cover), diversity numbers are given as single values. Single values for high cover are also given for the stations of streams with a wide range of cover.

Since no single index can treat all aspects of diversity, as we discuss in depth below in the discussion section, we have developed a combination diversity index as a mean of:

1. $Diversity_H = \ln\left(N - \frac{1}{N}\right) \sum_{n=1}^{s} (n_i \ln n_i)$ (Shannon's Index)

2. $Diversity_N = NS$

3. $Diversity_1 = \sum_{n=1}^{s} \ln(n_i + 1)$

4. $Diversity_2 = \sum_{n=1}^{s} \left[\left(\frac{N - n_i}{N} \right) (n_i \ln n_i) \right]$

Combination Diversity Vs. Canopy

PROVINCE = PIEDMONT

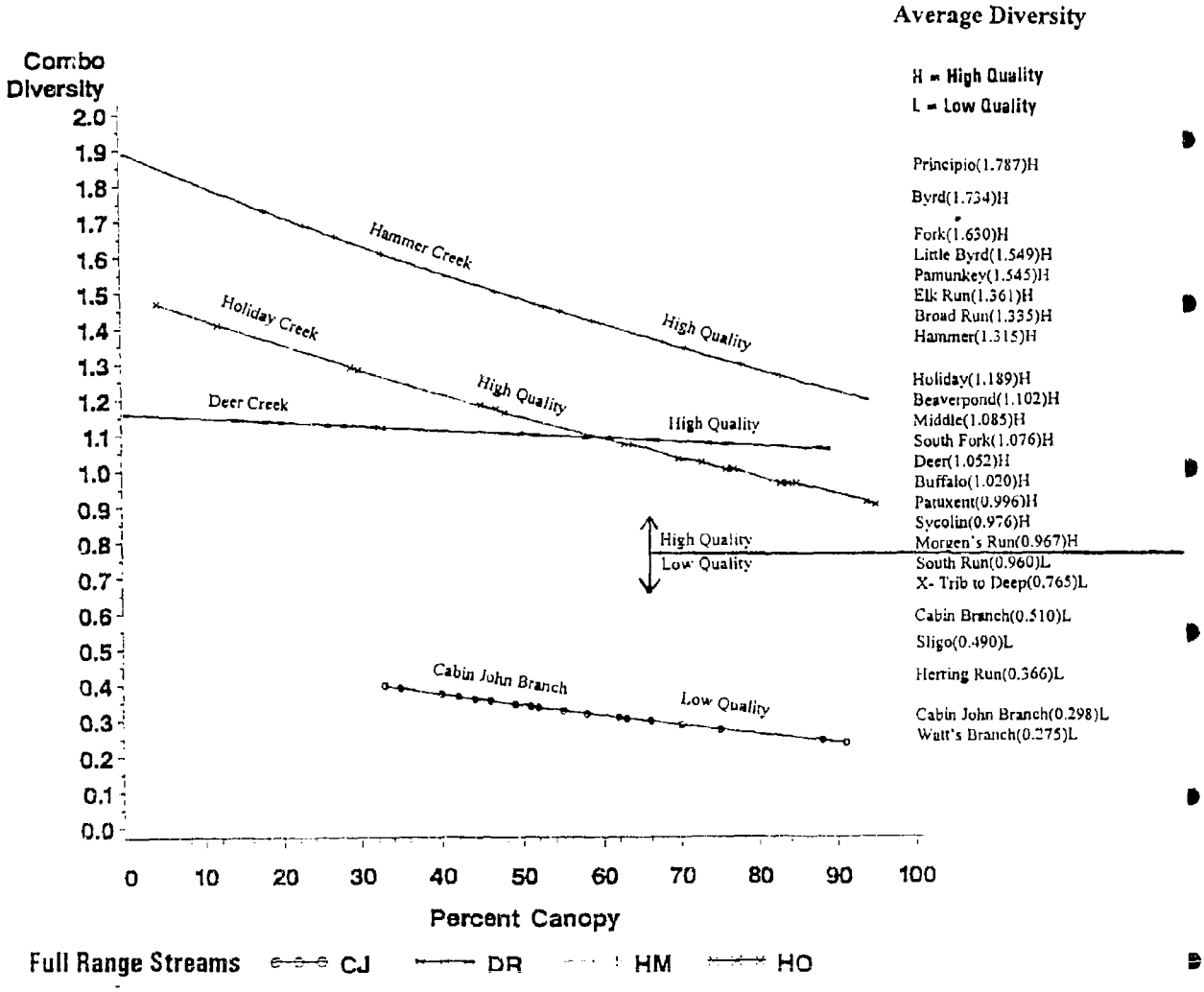

Figure 14. Exponentially regressed curves representing diversity as a function of tree canopy cover for those streams having a full range of canopy. Average diversity is the mean of the actual points for those sites having >66% canopy in all streams. Piedmont Province.

Combination Diversity Vs. Canopy

PROVINCE=COASTAL

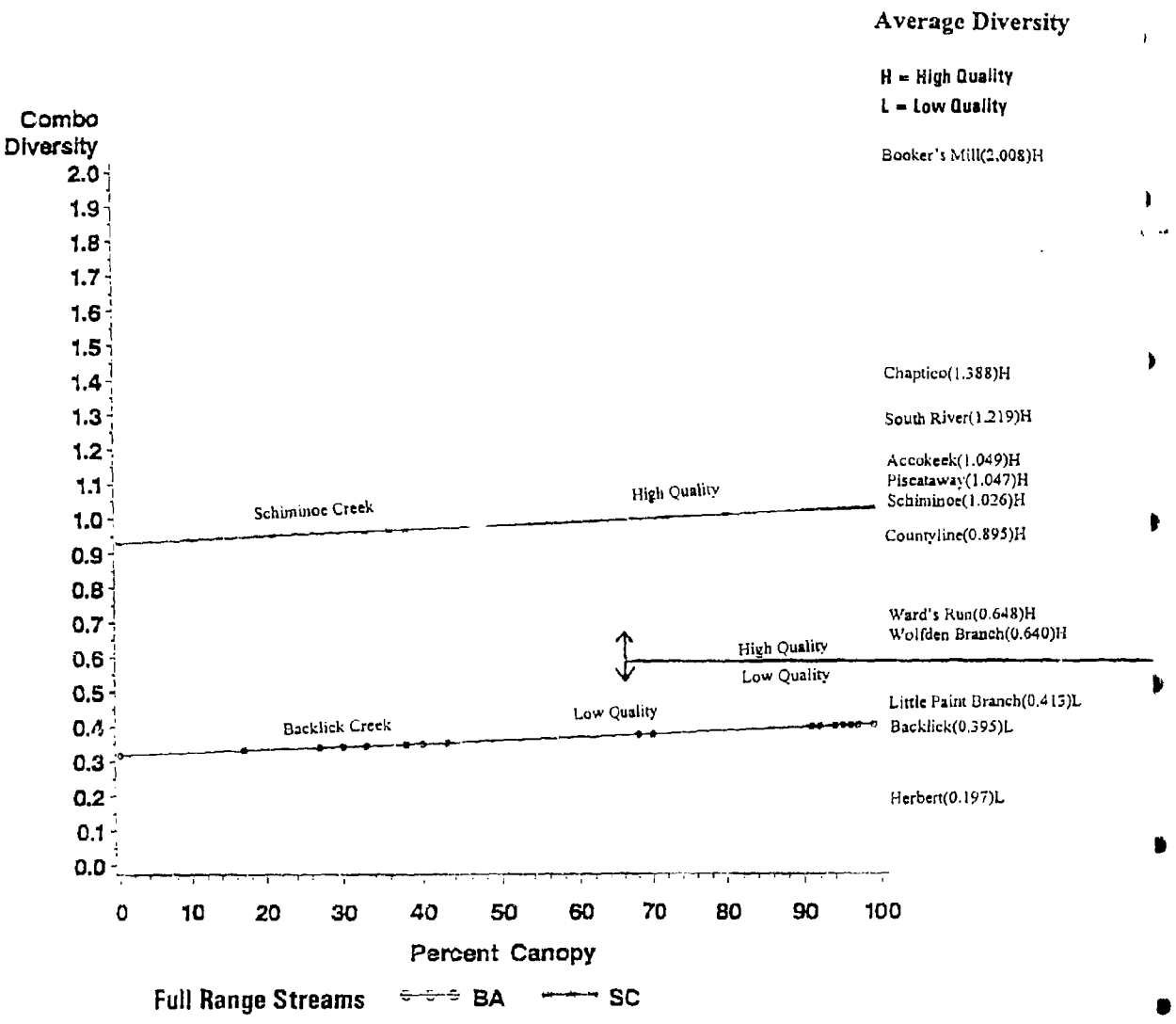

Figure 15. Exponentially regressed curves representing diversity as a function of tree canopy cover for those streams having a full range of canopy. Average diversity is the mean of the actual points for those sites having >66% canopy in all streams. Coastal Province.

Where N= total number of individuals, n = number of individuals of any one species and S= the total number of species. All indices were scaled to be weighted equally in the combination index. Shannon's diversity (1) emphasizes richness and evenness; $Diversity_N$ (2), a simple measure of total species times the total number of plants, emphasizes abundance and richness; $Diversity_1$ (3) emphasizes abundance and to a lesser extent evenness; and $Diversity_2$ (4) includes all three parameters. A mean of all four indices (scaled to the range for Shannon's) weights abundance and richness almost equally with less, but still important emphasis on evenness. For each stream, this index is plotted both as a function of cover and as a single number for solely high cover streams (Figures 14 and 15). This combination set of diversity indices separates all reference streams into their appropriate high and low categories.

DISCUSSION

Although a simple randomized cumulative count of macrophyte species number begins to separate most high quality from low quality streams with as few as 5-10 stations, in marginal cases larger numbers are required. Many marginal cases can be further separated by surveying more than 20 stations, perhaps 25-30 stations, in order to reach full saturation of species. In general, however, a full species count to more than 15-20 stations may be more time-consuming than the indicator species method or diversity methods discussed below.

Higher quality streams require a greater number of stations to bring the saturation curves to saturation and maximize diversity. However, this is not likely to affect the rating of a

particular high quality stream. For the Coastal Plain and Piedmont Provinces, it is perhaps best to simply establish 20 stations as a minimum requirement. In order to determine the degree of high quality (i.e., moderately high to extremely high) for baseline studies, more sites will be necessary to reach saturation.

In the reference stream data set that we have presented in this report, based on State surveys, two sets of macrophyte indicator species were developed. One of these sets includes four species that are very common in high quality streams and tend to significantly reduce in numbers of plants with stream degradation; the other set of seven species of more moderate abundance are those species that are likely to be totally lost with stream degradation. A plot of numbers of individuals of the first set against numbers of species of the second set has provided a strong differentiation of all high quality and low quality streams. This is shown in both the Coastal Plain and Piedmont reference sets (Figures 12 and 13).

A significant feature of this indicator species bioassay is the apparent acceptance of lower quality streams as being unimpaired in the Coastal Plain as compared to the Piedmont Province. Several workers in the field have already noted this feature as an inevitable consequence of denser population and greater industrialization (Primrose, 1994; Silvia, 1994). While it might be argued that this is a pre-Columbian characteristic of the Coastal Plain Province that distinguishes this province from the Piedmont, we point out that Booker's Mill Stream, well out on the Coastal Plain, has the highest indicator species rating of all the streams we surveyed. Indeed, whether or not the two provinces could have been differentiated on the basis of their

stream's macrophyte community and population structure in pre-Columbian times is questionable. However, if the provinces are not separated in a bioassay, then many Coastal Plain stream qualities must effectively be lowered.

The single low quality stream that rates relatively high on the indicator species scale in the Piedmont data set, South Run in Fauquier County Virginia, has been rated as only "moderately impaired" by the state. The single high quality stream that rates lowest on the indicator species scale in the Coastal Plain is Wolfden Branch in Prince Georges and Charles Counties of Maryland. A careful re-survey of the drainage basin of this stream has located a sewage sludge basin that was installed following the original rating but about six months before our survey. Thus, a lowering of water quality is suspect for this case.

It is apparent from this presentation that an indicator species test as a macrophyte stream bioassay provides nearly as much information as the more complex biodiversity measures. An experienced two person team can carry out the entire field bioassay process for a typical stream in a day using the biodiversity measures, including data entry and analysis with a lap top computer in the field or back in the laboratory. This contrasts with several days necessary to compile data for equivalent, previously used bioassay techniques. Using indicator species measures, on the other hand, would require only several hours in the field. The analysis could be completed on site with a hand calculator.

The complex macrophyte biodiversity analysis uses several indicators as a "combination index" that considers species abundance and richness essentially equally, and includes evenness to a lesser degree. The combination index separates all of our reference streams into high and low quality categories with the intermediate streams being appropriately placed. The pre-programmed analysis can be quickly carried out on a lap top computer. While this procedure is undoubtedly desirable to characterize a drainage basin or as a backup analysis in marginal cases, the additional time required to collect and process data is probably unwarranted for routine work.

To examine the comparability of the "very rapid" eleven indicator species method with the full macrophyte biodiversity method described, we have plotted combination diversity (x5 for scaling) as a function of combined indicator species in figure 16. The two types of indicator species were weighted equally in this plot. There is clearly a direct relationship between the two measures. However, equally important, the same reference streams fall into their respective high and low categories, and the questionable or intermediate streams are the same, regardless of method.

CONCLUSIONS

The community structure of stream macrophytes, primarily wetland obligate and facultative flowering plants, demonstrates patterns of community structure and diversity that are similar to those of other organisms that have been developed as bioassays of stream degradation. Unlike organisms used in other bioassays, macrophytes are considerably easier to identify and to

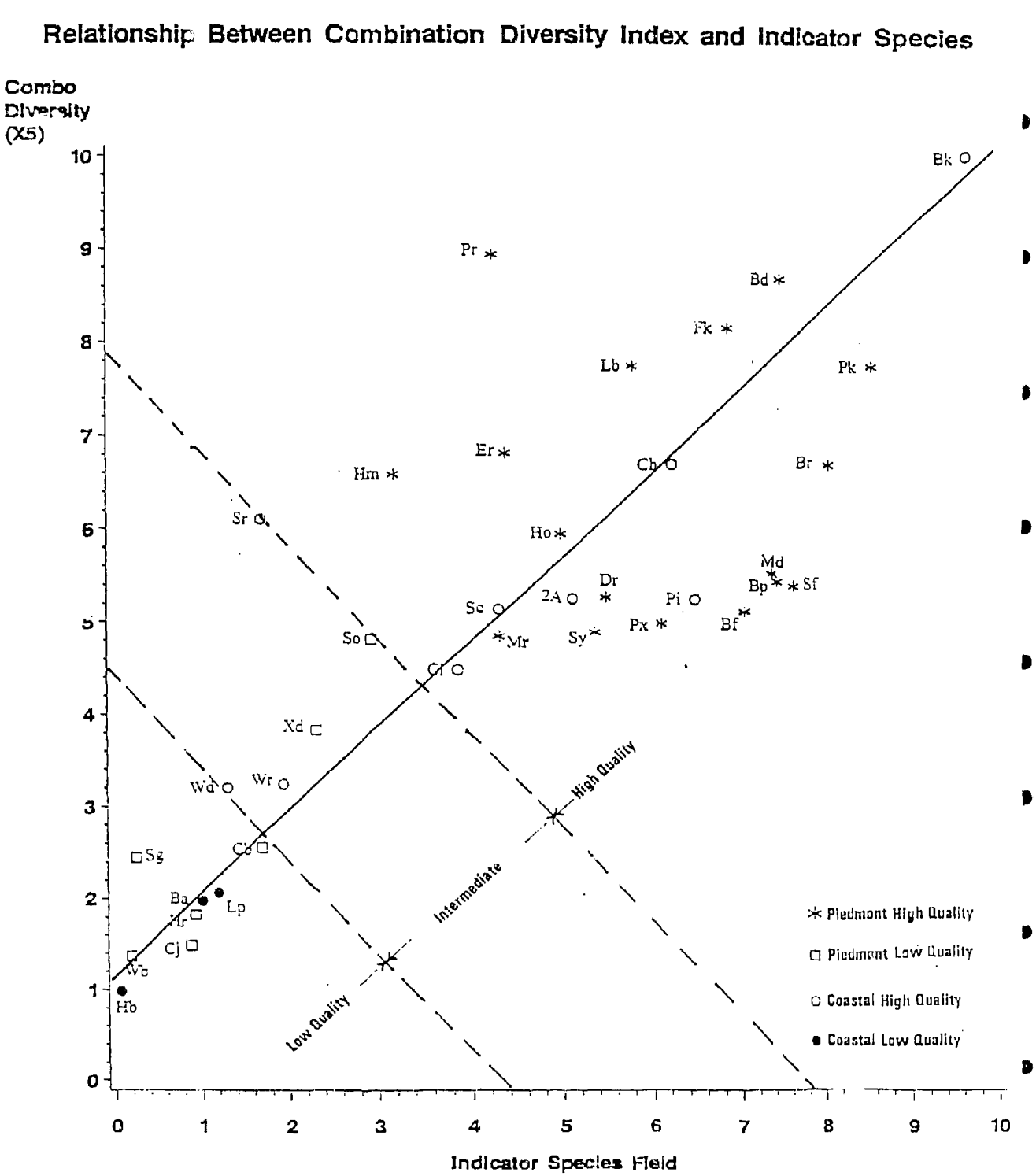

Figure 16. Correspondence between combination species diversity and indicator species for Piedmont and Coastal Plain Provinces.

quantify in regards to abundance. Simpler measures, such as using the number of macrophyte species and measuring the abundance and occurrence of limited numbers of index species, provide greater water quality differentiation.

In this study of the stream macrophytes of the Coastal and Piedmont Geomorphological Provinces of the Chesapeake Bay Watershed, we have demonstrated with reference streams, derived from state surveys, that a complex diversity measure can provide secure separation of these streams in accordance with their pre-rated character. This is accomplished through a rapid bioassay (1 to 1.5 day process) that can be carried out by two trained technicians. We have also demonstrated that an even more rapid bioassay (a few hours) can be carried out by the same team using a set of eleven indicator species. This "very rapid" bioassay will apply to the vast majority of the streams under survey. Only the borderline streams or those under considerable dispute would require the entire assay.

During 1995, the streams of all of the military bases of the Chesapeake Bay Watershed to which we have access will be surveyed and rated using the process described in this report. The Valley and Ridge Province, the field work for which was completed in 1994, will be analyzed and added to the repertory. As time permits, additional provinces will be added to the data set and additional reference streams will be added to the more limited Coastal Plain Province.

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APPENDIX A.

NUMBER KEY FOR STREAMS SURVEYED.

APPENDIX A. STREAM KEY FOR STREAMS PLOTTED ON FIGURES 12, 13, AND 16.

HQ= High Quality Streams
LQ= Low Quality Streams

HQ 1.	(SB)	Stafford Meadow Brook- Lackawanna Co., PA
HQ 2.	(NP)	Nescopeck Creek- Luzerne Co., PA
HQ 3.	(OL)	Oley Creek- Luzerne Co., PA
HQ 4.	(LC)	Little Catawissa Creek- Schuylkill Co., PA
HQ 5.	(DA)	Dark Creek- Schuylkill Co., PA
HQ 6.	(RO)	Roaring Creek- Columbia Co., PA
HQ 7.	(HN)	Honey Creek- Mifflin Co., PA
HQ 8.	(ST)	Stoney Creek- Huntingdon Co., PA
HQ 9.	(SH)	Shaver Creek- Huntingdon Co., PA
HQ 10.	(DK)	Duck Creek- Frederick Co., PA
HQ 11.	(PA)	Passage Creek- Shenandoah Co., PA
HQ 12.	(Lk)	Little Back Creek- Bath Co., VA
HQ 13.	(PM)	Pounding Mill Creek- Alleghany Co., VA
HQ 14.	(SM)	St. Mary's Creek- Augusta Co., VA
HQ 15.	(HM)	Hammer Creek- Lancaster Co., PA
HQ 16.	(MD)	Middle Creek- Adams Co., PA
HQ 17.	(DR)	Deer Creek- Baltimore Co., MD (PA Border)
HQ 18.	(PR)	Principio Creek- Cecil Co., MD
HQ 19.	(MR)	Morgan Run- Carrol Co., MD
LQ 20.	(HR)	Herring Run- Baltimore Co., MD
HQ 21.	(PX)	Patuxent Creek- Howard/Montgomery Co., MD
LQ 22.	(CB)	Cabin Branch- Montgomery Co., MD
LQ 23.	(SG)	Sligo Creek- Montgomery Co., MD
HQ 24.	(SY)	Sycolin Creek- Loudoun Co., VA
LQ 25.	(WB)	Watt's Branch- Montgomery Co., MD
LQ 26.	(CJ)	Cabin John Branch- Montgomery Co., MD
HQ 27.	(BR)	Broad Run- Loudoun Co., VA
HQ 28.	(SF)	South Fork- Loudoun Co., VA
LQ 29.	(SO)	South Run- Faquier Co., VA
HQ 30.	(JO)	John's Creek- Craig Co., VA
HQ 31.	(ER)	Elk Run- Faquier Co., VA
HQ 33.	(PK)	Pamunky Creek- Ornge Co., VA
HQ 34.	(FK)	Fork Creek- Louisa Co., VA
HQ 35.	(BD)	Byrd/Venable Creek- Fluvanna Co., VA
HQ 36.	(LB)	Little Byrd/Peter's Creek- Goochland Co., VA
HQ 37.	(HO)	Holiday Creek- Buckingham/Appomattox Co., VA
HQ 38.	(BP)	Beaverpond Creek- Amelia Co., VA
HQ 39.	(BF)	Buffalo Creek- Prince Edward Co., VA

LQ 40.	(XD)	X-trib to Deep Creek- Nottoway Co., VA
LQ 41.	(HB)	Herbert Run- Anne Arundel Co., MD
LQ 42.	(LP)	Little Paint Branch- Prince George's Co., MD
LQ 46.	(BA)	Backlick Creek- Fairfax Co., VA
HQ 47.	(WD)	Wolfden Branch- Prince George's Co., MD
HQ 48.	(CL)	Countyline Creek- Prince George's Co., MD
HQ 49.	(WR)	Ward's Run- Charles Co., MD
HQ 50.	(CH)	Chaptico Creek- St. Mary's Co., MD
HQ 51.	(2A)	Accokeek Creek- Stafford Co., VA
HQ 53.	(SR)	South River- Caroline Co., VA
HQ 55.	(PI)	Piscataway Creek- Essex Co., VA
HQ 56.	(BK)	Booker's Mill- Richmond, Co., VA
HQ 57.	(SC)	Schiminoe Creek

APPENDIX B.

NUMBER KEY TO SPECIES AND COORDINATES FOR FIGURE 8 AND FIGURE 9.

APPENDIX B. NUMBER KEY TO SPECIES AND COORDINATES FOR SPECIES PLOTTED ON FIGURES 8 AND 9. SPECIES LIST FOR PIEDMONT AND COASTAL GEOZONES.

C = Coastal species retained for calculation of diversity indices.
P = Piedmont species retained for calculation of diversity indices.
* = Combined with 1-3 species of same genus for P and/or C.

			GENUS	SPECIES	HIMEAN	HIPERCENT	LOMEAN	LOPERCENT
	1		Agaratina	altissima	0.00000	0.0000	0.3	10
	2		Ambrosia	artemisiifolia	0.00000	0.0000	0.2	10
	3		Artemisia	vulgaris	0.00000	0.0000	2.0	10
	4		Cyperus	oderatus	0.00000	0.0000	0.6	20
	5		Cyperus	rivularis	0.00000	0.0000	0.1	10
	6		Eupatorium	rugosum	0.00000	0.0000	0.3	10
	7		Zizania	aquatica	0.00000	0.0000	0.1	10
	8		Athyrium	Filix-femina	0.03846	3.8462	0.0	0
C	9		Ceratophyllum	demersum	0.03846	3.8462	0.0	0
	10		Chenopodium	ambrosioides	0.03846	3.8462	0.0	0
C	11		Cyperus	erythrorhizos	0.03846	3.8462	2.2	60
	12		Lactuca	biennis	0.03846	3.8462	0.0	0
P	13		Lycopus	americanus	0.03846	3.8462	0.0	0
	14		Orontium	aquaticum	0.03846	3.8462	0.0	0
	15		Phytolacca	americana	0.03846	3.8462	0.0	0
	16		Ranunculus	septentrionalis	0.03846	3.8462	0.0	0
	17		Samolus	parviflorus	0.03846	3.8462	0.0	0
	18		Scirpus	atrovirens	0.03846	3.8462	0.0	0
	19		Scirpus	cyperinus	0.03846	3.8462	0.0	0
	20		Scutellaria	integrifolia	0.03846	3.8462	0.0	0
	21		Verbascum	thapsus	0.03846	3.8462	0.0	0
	22		Xanthium	strumarium	0.03846	3.8462	0.0	0
	23		Ampelamus	albidus	0.07692	3.8462	0.0	0
	24		Cicuta	maculata	0.07692	3.8462	0.0	0
	25		Heuchera	americana	0.07692	3.8462	0.0	0
	26		Rorippa	palustris	0.07692	7.6923	0.0	0
	27		Solanum	carolinense	0.07692	3.8462	0.0	0
	28		Typha	latifolia	0.07692	3.8462	0.0	0
	29		Rhynchospora	macrostachya	0.11538	3.8462	0.0	0
	30		Solidago	speciosa	0.11538	3.8462	0.0	0
	31		Laportea	canadensis	0.15385	3.8462	0.0	0
	32		Amaranthus	spinosus	0.19231	3.8462	0.0	0
	33		Aster	puniceus	0.19231	3.8462	0.0	0
	34		Bidens	laevis	0.19231	3.8462	0.0	0
	35		Carex	tribuloides	0.19231	3.8462	0.0	0
	36		Cerastium	glomeratum	0.19231	3.8462	0.0	0
	37		Chenopodium	album	0.19231	3.8462	0.0	0
	38		Juncus	canadensis	0.19231	3.8462	0.0	0
	39		Lespedeza	cuneata	0.19231	3.8462	0.0	0
	40		Myosoton	aquaticum	0.19231	3.8462	0.0	0
P	41	*	Potamogeton	pusillus	0.19231	3.8462	0.0	0
	42		Sambucus	canadensis	0.19231	3.8462	0.0	0
	43		Silene	antirhina	0.19231	3.8462	0.0	0
	44		Solidago	caesia	0.19231	3.8462	0.0	0

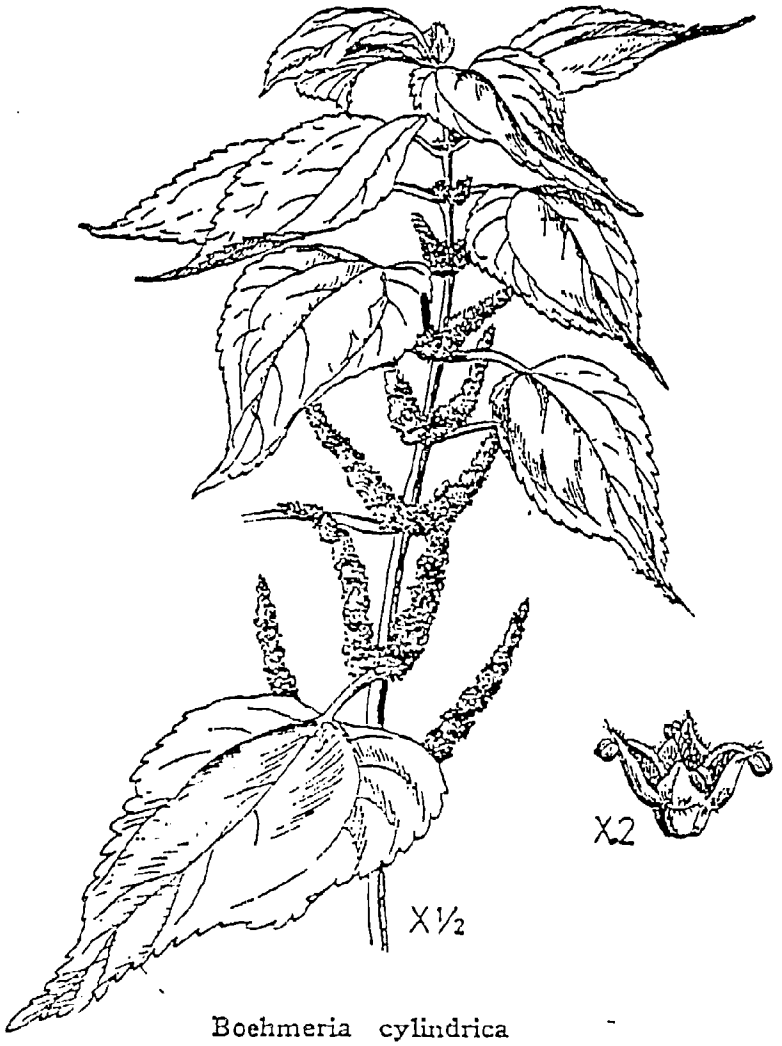
	45		Valeriana	pauciflora	0.19231	3.8462	0.0	0
	46		Ampelopsis	arborea	0.23077	7.6923	0.0	0
	47		Aster	vimineus	0.23077	11.5385	0.0	0
P	48		Onoclea	sensibilis	0.23077	11.5385	0.0	0
	49		Physalis	heterophylla	0.23077	3.8462	0.0	0
	50		Pontedaria	cordata	0.23077	3.8462	0.0	0
	51		Ranunculus	abortiuus	0.23077	3.8462	0.0	0
P	52		Lobelia	inflata	0.26923	11.5385	0.0	0
CP	53	*	Polygonum	pensylvanicum	0.26923	11.5385	1.0	20
	54		Asarum	canadense	0.30769	3.8462	0.0	0
P	55		Lobelia	siphilitica	0.30769	11.5385	0.0	0
	56		Rudbeckia	triloba	0.30769	7.6923	0.9	30
	57		Verbena	urticifolia	0.34615	11.5385	0.0	0
	58		Aster	novae-angliae	0.38462	3.8462	0.0	0
	59		Dianthus	barbatus	0.38462	3.8462	0.0	0
	60		Linum	striatum	0.38462	3.8462	0.0	0
	61		Lycopus	rubellus	0.38462	3.8462	0.0	0
P	62		Mentha	spicata	0.38462	3.8462	0.0	0
	63		Phryma	leftostachya	0.38462	3.8462	0.0	0
CP	64	*	Potamogeton	diversifolius	0.38462	3.8462	0.0	0
	65		Rudbeckia	hirta	0.38462	3.8462	0.0	0
P	66	*	Solidago	erecta	0.38462	3.8462	0.0	0
P	67		Carex	squarrosa	0.42308	7.6923	0.0	0
	68		Nuphar	luteum	0.42308	7.6923	0.0	0
	69		Smilacinia	racemosa	0.42308	11.5385	0.0	0
	70		Symplocarpus	foetidus	0.42308	23.0769	0.0	0
C	71		Triadenum	Walteri	0.42308	11.5385	0.0	0
	72		Conoclinium	coelestinum	0.46154	11.5385	0.0	0
	73		Alliaria	petiolata	0.50000	11.5385	0.0	0
	74		Amaranthus	hybridus	0.50000	7.6923	0.0	0
C	75		Carex	intumescens	0.50000	15.3846	0.0	0
	76		Elephantopus	carolinianus	0.50000	3.8462	0.0	0
	77		Galium	triflorum	0.50000	7.6923	0.0	0
	78		Lespedeza	striata	0.50000	11.5385	0.0	0
	79		Phlox	paniculata	0.50000	3.8462	0.0	0
P	80		Fontinalis	flaccidus	0.57692	3.8462	0.0	0
	81		Lysimachia	nummularia	0.57692	11.5385	0.0	0
C	82		Nitella	flexis	0.57692	3.8462	0.0	0
	83		Sisyrinchium	arenicola	0.57692	3.8462	0.0	0
	84		Polystichum	acrostichoides	0.65385	3.8462	0.6	20
P	85		Agrimonia	parviflora	0.69231	19.2308	0.0	0
	86		Circaea	lutetiana	0.69231	11.5385	0.0	0
P	87	*	Solidago	rugosa	0.69231	11.5385	0.0	0
	88		Urtica	dioica	0.69231	11.5385	0.0	0
	89		Anagallis	arvensis	0.73077	7.6923	0.0	0
	90		Rotala	ramosior	0.76923	3.8462	0.0	0
	91		Teucrium	canadense	0.76923	7.6923	0.0	0
	92		Veronica	Anagallis-aquatica	0.76923	7.6923	0.0	0
	93		Juncus	scirpoides	0.84615	3.8462	0.0	0
	94		Lycopersicon	esculentum	0.84615	3.8462	0.0	0
P	95		Barbarea	verna	0.88462	15.3846	0.0	0
	96		Geum	canadense	0.88462	11.5385	0.0	0
P	97		Mentha	piperita	0.88462	7.6923	0.0	0

P 98	*	Potamogeton	crispus	0.96154	3.8462	0.0	0
C 99		Aster	undulatus	1.00000	7.6923	0.0	0
C 100		Carex	alata	1.07692	23.0769	0.0	0
P 101	*	Solidago	canadensis	1.07692	11.5385	0.0	0
102		Verbesina	occidentalis	1.11538	3.8462	0.0	0
103		Rumex	obtusifolius	1.19231	19.2308	0.7	50
104		Verbena	hastata	1.19231	7.6923	0.0	0
C 105		Saururus	cernuus	1.23077	7.6923	0.0	0
106		Solanum	dulcamara	1.26923	7.6923	0.0	0
P 107		Verbesina	alternifolia	1.26923	15.3846	0.0	0
P 108		Sagittaria	latifolia	1.30769	26.9231	0.0	0
109		Carex	seorsa	1.34615	3.8462	0.0	0
P 110		Ambrosia	trifida	1.38462	15.3846	0.0	0
P 111	*	Barbarea	vulgaris	1.38462	11.5385	1.1	
112		Glecoma	hederacea	1.38462	11.5385	0.0	0
C 113		Carex	laevivaginata	1.46154	19.2308	0.0	0
CP114		Ludwigia	decurrens	1.46154	11.5385	0.0	0
P 115		Scirpus	polyphyllus	1.46154	15.3846	0.0	0
116		Arisaema	triphyllum	1.53846	7.6923	0.0	0
C 117		Woodwardia	areolata	1.57692	30.7692	0.0	0
CP118		Stellaria	media	1.69231	19.2308	7.7	60
P 119		Aster	dumosum	1.73077	23.0769	0.0	0
CP120		Carex	crinita	1.73077	30.7692	0.0	0
CP121		Scutellaria	lateriflora	1.80769	26.9231	0.0	0
CP122		Gratiola	virginiana	1.88462	23.0769	0.0	0
CP123		Ludwigia	alternifolia	2.00000	11.5385	0.5	10
C 124		Triadenum	virginicum	2.07692	3.8462	0.0	0
CP125		Juncus	marginatus	2.19231	50.0000	0.7	20
126		Cyperus	strigosus	2.23077	11.5385	0.0	0
P 127		Galium	Mollugo	2.23077	23.0769	0.5	10
P 128		Heteranthera	reniformis	2.38462	7.6923	0.0	0
P 129	*	Potamogeton	sp.	2.38462	11.5385	0.0	0
P 130		Cryptotaenia	canadensis	2.42308	23.0769	0.0	0
CP131		Alisma	subcordatum	2.50000	42.3077	0.0	0
P 132		Carex	frankii	2.50000	26.9231	0.0	0
CP133		Chelone	glabra	2.50000	23.0769	0.1	0
P 134		Thalictrum	revolutum	2.53846	38.4615	0.4	10
P 135		Plantago	rugelli	2.65385	42.3077	0.8	20
P 136		Prunella	vulgaris	2.65385	26.9231	0.0	0
P 137	*	Solidago	sp.	2.65385	11.5385	0.0	0
CP138		Juncus	effusus	2.76923	50.0000	0.0	0
CP139		Peltandra	virginica	2.76923	23.0769	0.0	0
P 140	*	Viola	papilionacea	2.76923	34.6154	0.0	0
C 141		Potamogeton	epihydrus	2.88462	11.5385	0.0	0
P 142		Penthorum	sedoides	3.26923	11.5385	0.0	0
P 143		Perilla	frutescens	3.26923	26.9231	1.0	30
CP144		Cardamine	hirsuta	3.50000	34.6154	1.9	60
P 145		Rudbeckia	laciniata	3.53846	23.0769	0.4	10
P 146		Gratiola	neglecta	3.76923	23.0769	0.0	0
CP147		Lobelia	cardinalis	3.84615	57.6923	0.8	10
148		Bidens	cernua	4.19231	30.7692	0.0	0
P 149		Commelina	virginica	4.19231	34.6154	0.0	0
150		Erigeron	annuus	4.23077	3.8462	0.0	0

CP151		Dulichium	arundinaceum	5.07692	23.0769	0.0	0
CP152		Galium	obtusum	5.26923	69.2308	0.0	0
P 153		Equisetum	arvense	5.46154	15.3846	0.0	0
P 154	*	Potamogeton	foliosus	5.769	3.846	0.0	0
P 155		Stellaria	pubera	6.346	11.538	0.5	10
P 156	*	Viola	sp.	6.346	38.462	33.2	90
P 157		Commelina	communis	6.769	11.538	9.7	50
P 158		Mentha	arvensis	7.038	19.231	1.5	10
CP159		Nasturtium	officianale	7.077	26.923	0.0	0
P 160		Mimulus	ringens	7.346	46.154	0.0	0
161		Sedum	ternatum	7.692	3.846	0.0	0
P 162		Proserpinaca	palustris	7.731	15.385	0.0	0
CP163		Galium	tinctorium	7.923	50.000	0.0	0
CP164		Eupatorium	perfoliatum	8.385	61.538	0.1	10
P 165		Acalypha	rhomboidea	8.462	50.000	2.7	60
CP166	*	Polygonum	punctatum	8.462	26.923	31.9	70
P 167		Polygonum	virginianum	9.423	42.308	2.0	20
CP168		Mimulus	alatus	9.692	61.538	7.6	50
CP169		Polygonum	arifolium	9.923	53.846	1.7	10
CP170		Bidens	tripartita	13.000	69.231	1.9	40
CP171		Carex	lurida	13.769	65.385	0.0	0
CP172		Aster	prenanthoides	13.885	53.846	18.4	50
CP173		Senecio	aureus	14.462	30.769	0.0	0
CP174		Sparganium	sp. (limp leaf)	14.885	42.308	0.0	0
CP175		Aster	lateriflorus	15.808	65.385	14.1	10
P 176	*	Viola	palmata	16.038	65.385	0.0	0
CP177		Lemna	minor	16.538	11.538	0.0	0
CP178		Bidens	frondosa	18.769	73.077	4.3	40
CP179	*	Polygonum	cespitosum	19.538	23.077	119.4	90
CP180	*	Eupatoriadelphus	sp.	22.346	76.923	3.1	70
CP181		Lycopus	virginicus	23.308	88.462	2.6	70
P 182		Elodea	canadensis	23.962	15.385	1.0	10
CP183		Aster	divaricatus	24.769	57.692	1.2	20
CP184		Thelypteris	novaeboracensis	25.462	57.692	0.6	10
CP185		Sparganium	americanum	26.346	26.923	0.0	0
CP186		Aster	simplex	29.231	80.769	40.2	70
CP187		Polygonum	sagittum	35.500	100.000	16.4	50
P 188		Justicia	americana	42.692	15.385	0.0	0
CP189		Eleocharis	obtusa	46.846	61.538	0.2	20
CP190		Lindernia	dubia	50.192	61.538	0.5	10
CP191		Fontinalis	novae angliae	51.115	46.154	0.0	0
CP192		Hypericum	mutilum	57.192	92.308	4.4	30
CP193		Ludwigia	palustris	57.462	80.769	24.0	80
CP194		Impatiens	capensis	64.538	92.308	6.9	50
CP195	*	Callitriche	sp.	73.885	50.000	0.0	0
CP196		Boehmeria	cylindrica	102.308	100.000	49.6	90
CP197		Pilea	pumila	157.577	92.308	39.2	100
CP198		Murdannia	keisak	233.692	42.308	0.5	10

APPENDIX C.

INDICATOR SPECIES PRIMARILY LOSING NUMBERS OF INDIVIDUALS.

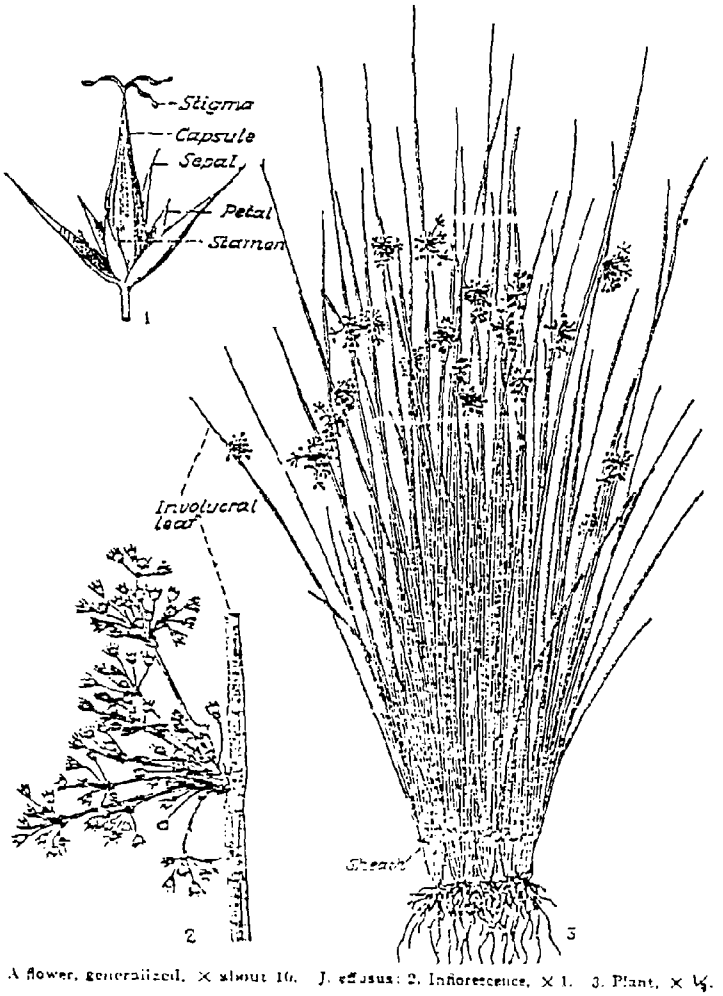


Boehmeria cylindrica

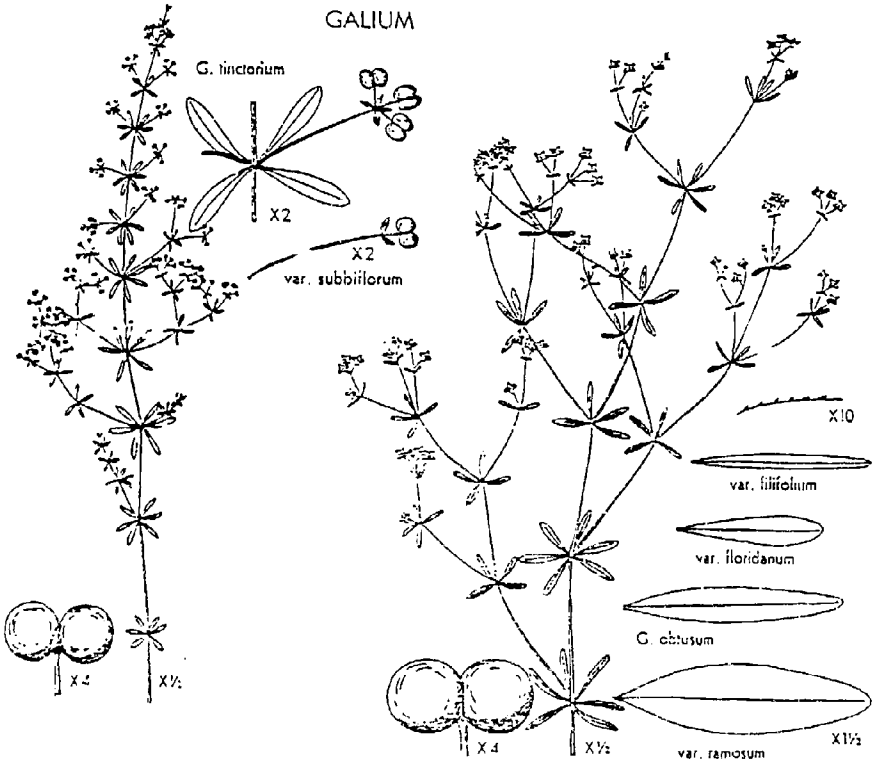
Polygonum sagittatum: a, portions of plant; b, portion of stem; c, flower cluster; d, flower, opened out; e, achene (From Correll and Correll, 1972)

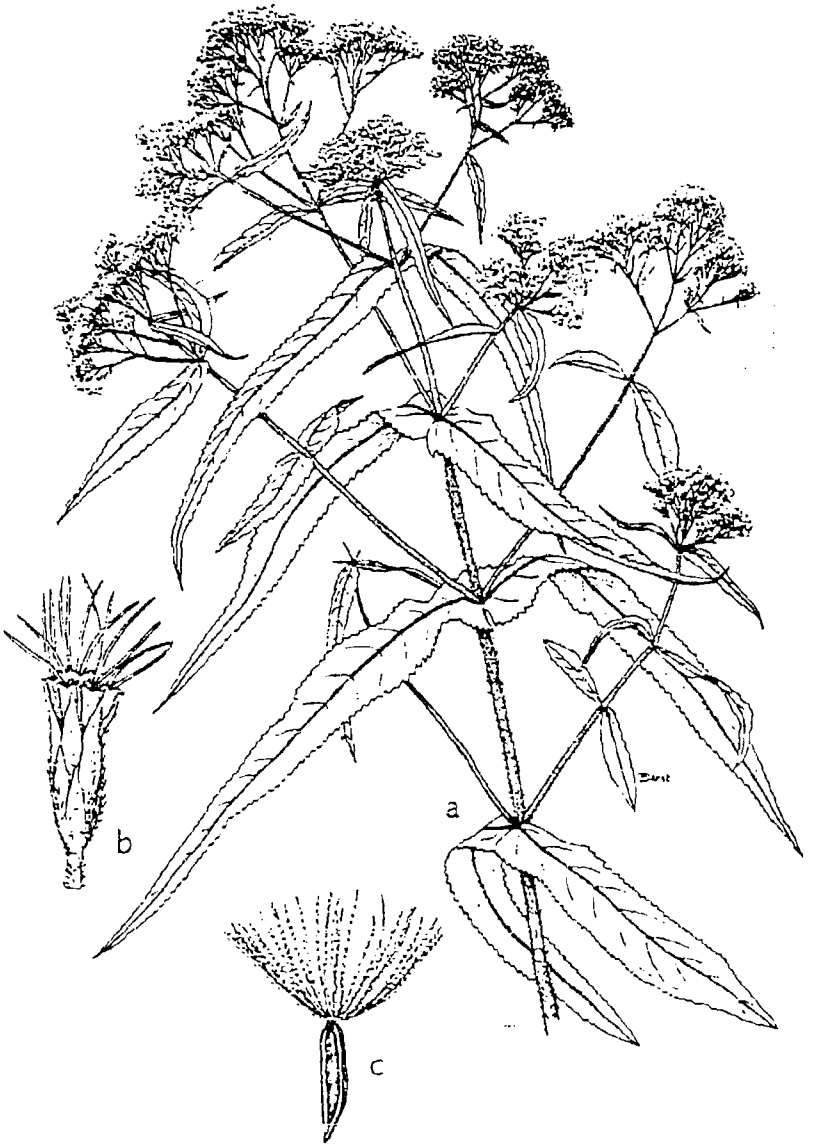
Lindernia dubia

JUNCUS



1. A flower, generalized, × about 10. J. effusus: 2. Inflorescence, × 1. 3. Plant, × ½.


GALIUM
G. tinctorium
X2
X2
var. subbiiflorum
X4
X½
X10
var. filifolium
var. floridanum
G. obtusum
X4
X½
var. ramosum
X1½

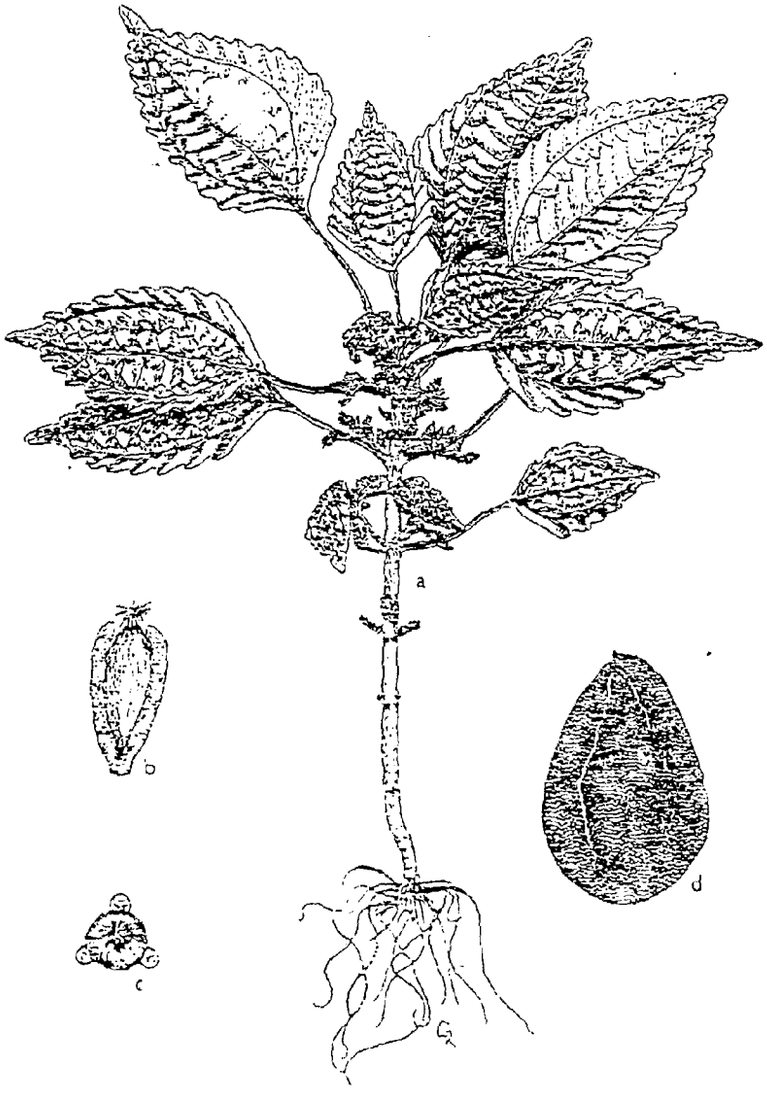


Eupatorium perfoliatum: a, top of plant; b, head; c, achene.

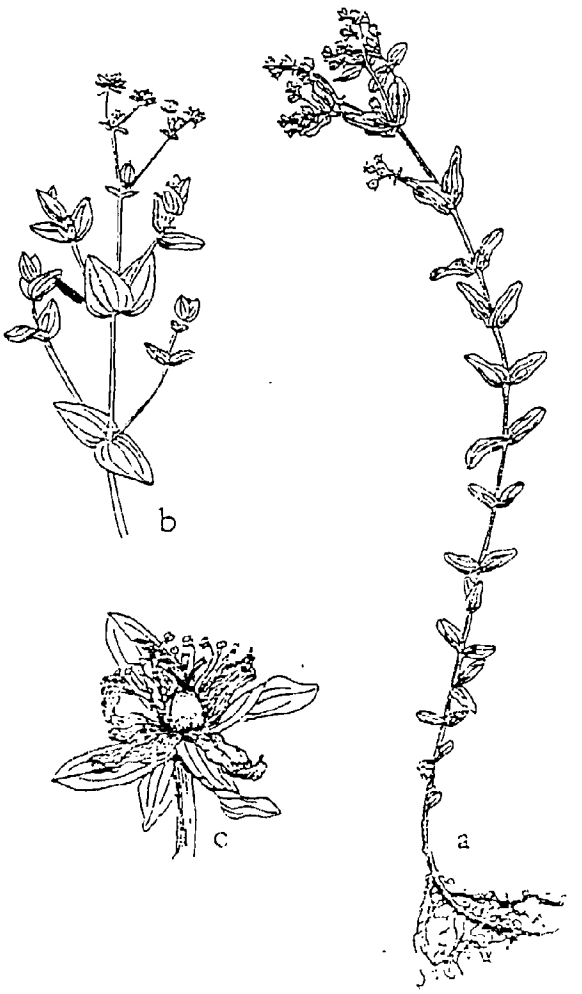
Aster lateriflorus

APPENDIX D.

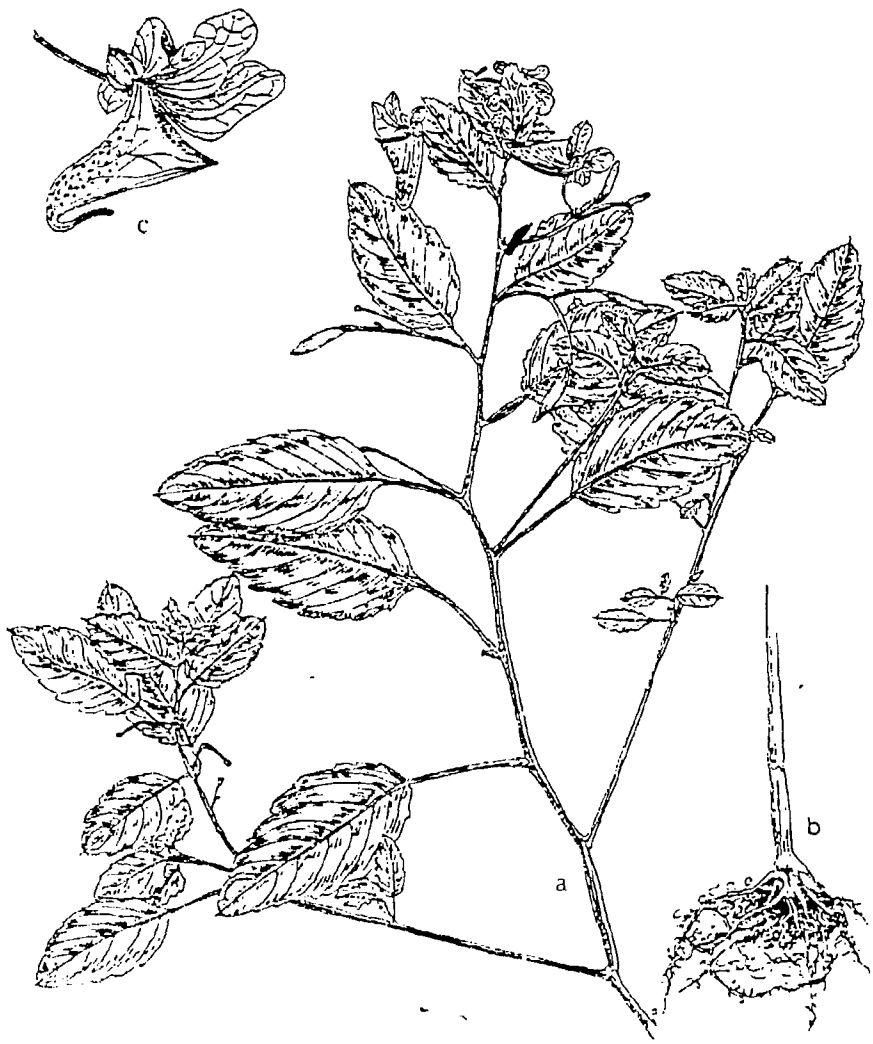
INDICATOR SPECIES PRIMARILY LOSING FREQUENCY.



Pilea pumila: a, habit; b, staminate flower, side view; c, pistillate flower, from above; d, fruit.



Hypericum mutilum: a, habit; b, top of plant; c, flower. (From Correll and Correll, 1972)



Impatiens capensis: a, flowering branch; b, base of plant; c, flower. (From Correll and Correll, 1972)